| Ref # | Hits | Search Query | DBs | Default Operator | Plurals | Time Stamp |
|---|---|---|---|---|---|---|
| L1 | 18311 | gst or glutathione s transferase$1 | US-PGPUB; USPAT | ADJ | OFF | 2005/02/03 10:46 |
| L2 | 220 | l1 near5 (plant or soy or glycine) | US-PGPUB; USPAT | ADJ | OFF | 2005/02/03 10:57 |
| L3 | 206 | 2 and (shuffl$ or muta$10) | US-PGPUB; USPAT | ADJ | OFF | 2005/02/03 10:49 |
| L4 | 2700 | 1 same (shuffl$ or muta$10) | US-PGPUB; USPAT | ADJ | OFF | 2005/02/03 10:49 |
| L5 | 27 | 2 and 4 | US-PGPUB; USPAT | ADJ | OFF | 2005/02/03 10:49 |
| L6 | 92 | l1 near5 (soy or glycine) | US-PGPUB; USPAT | ADJ | OFF | 2005/02/03 10:58 |
| L7 | 5381 | 1 near5 (gene$1 or sequence$1) | US-PGPUB; USPAT | OR | OFF | 2005/02/03 10:59 |
| L8 | 19 | 6 and 7 | US-PGPUB; USPAT | OR | OFF | 2005/02/03 10:59 |

| Ref # | Hits | Search Query | DBs | Default Operator | Plurals | Time Stamp |
|---|---|---|---|---|---|---|
| L1 | 18311 | gst or glutathione s transferase$1 | US-PGPUB; USPAT | ADJ | OFF | 2005/02/03 10:46 |
| L2 | 220 | l1 near5 (plant or soy or glycine) | US-PGPUB; USPAT | ADJ | OFF | 2005/02/03 10:47 |
| L3 | 206 | 2 and (shuffl$ or muta$10) | US-PGPUB; USPAT | ADJ | OFF | 2005/02/03 10:49 |
| L4 | 2700 | 1 same (shuffl$ or muta$10) | US-PGPUB; USPAT | ADJ | OFF | 2005/02/03 10:49 |
| L5 | 27 | 2 and 4 | US-PGPUB; USPAT | ADJ | OFF | 2005/02/03 10:49 |

PGPUB-DOCUMENT-NUMBER: 20040185460

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040185460 A1

TITLE: Novel mixed lineage kinase (7) (mlk7) polypeptide polynucleotides encoding the same and methods of use thereof

PUBLICATION-DATE: September 23, 2004

INVENTOR-INFORMATION:

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APPL-NO: 10/ 478068

DATE FILED: April 20, 2004

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60293381 20010524 US

PCT-DATA:
APPL-NO: PCT/US02/16387
DATE-FILED: May 23, 2002
PUB-NO:
PUB-DATE:
371-DATE:
102(E)-DATE:

US-CL-CURRENT: 435/6, 435/194 , 435/235.1 , 435/320.1 , 435/325 , 435/69.1 , 536/23.2

ABSTRACT:

The present invention provides isolated mixed lineage kinase (7) (MLK) polynucleotides, expression vectors, host cells, isolated polypeptides, methods of producing polypeptides, isolated antibodies, compositions having the foregoing, methods for identifying a compound that binds a polypeptide or polynucleotide, and to methods for identifying a compound that modulates the activity of a poypeptide.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims priority to U.S. provisional application Serial No. 60/293,381 filed May 24, 2001, which is incorporated herein by reference in its entirety.

---------- KWIC ---------

Detail Description Paragraph - DETX (30):

[0056] The present invention also provides MLK7 variants having additional amino acid residues that result from use of specific expression systems. For example, use of commercially available vectors that express a desired polypeptide as part of a glutathione-S-transferase (GST) fusion product provides the desired polypeptide having an additional glycine residue at position -1 after cleavage of the GST component from the desired polypeptide. Variants that result from expression in other vector systems are also contemplated. Insertional variants also include fusion proteins wherein the amino terminus and/or the carboxy terminus of MLK7 is/are fused to another polypeptide. In addition, other fusion proteins comprising MLK7 or fragments thereof are contemplated by the invention. Numerous fusion partner proteins are well known to the skilled artisan.

Detail Description Paragraph - DETX (101):

[0120] ELISA Assay Protocol: Assays are performed in 96 well FluoroNunc Maxisorp ELISA plates coated with 10 .mu.g/ml GST-MKK4 (kinase dead mutant) diluted in Tris buffered saline (TBS). Coating is achieved by allowing the MKK4 substrate to stand in the wells for 16 hours at 4 C in a humidified chamber. After coating, excess buffer is aspirated, plates are washed 3 times with TBS containing 0.05% Tween 20 (v/v), and blocked with 3% BSA (w/v) in TBS-T (200 .mu.l/well) for 1 hour at 37 C. All subsequent incubations are carried out using 100 .mu.l/well volumes for one hour at 37 C in a humidified chamber. After blocking, plates are washed 3 times in TBS-T and TBS, respectively. The kinase reaction is performed in 20 mM HEPES, pH 7.4, 30 .mu.M ATP, 15 mM MgCl.sub.2, 1 mM DTT, 0.1 mM Na.sub.3VO.sub.4, 5 mM EGTA, and 25 mM .beta.-glycerophosphate (90 .mu.l/well) and 50 ng/ml GST-MLK7.sub.KD (10 .mu.l/well) for 30 minutes at 37 C. As a negative control, 0.5 M EDTA (30 .mu.l/well) is added to the reaction prior to incubation. Plates are washed 3 times in TBS-T and polyclonal antibody 226.1 (New England Biolabs), which detects phosphorylated MKK4, is added to wells at a [fraction (1/5000)] dilution in blocking buffer. After incubation, plates are washed and alkaline phosphatase conjugated goat anti-rabbit secondary antibody is added at a [fraction (1/2500)] dilution in blocking buffer. Following a 1-hour incubation and plate washing, 4-methyl umbelliferyl phosphate (0.2 mg/ml final concentration) is added to wells and allowed to develop for 45 minutes. The reaction is terminated with 0.5 M Na.sub.2HPO.sub.4 and read on a fluorescence plate reader at 360 nm excitation wavelength and 460 nm emission wavelength.

Detail Description Paragraph - DETX (102):

[0121] Optimization of ELISA: The ELISA is optimized with regard to substrate preference and coating concentration, primary antibody dilution, and development time for the fluorogenic substrate. Magnesium ion requirements, sensitivity to reductants and DMSO can also be evaluated. Both MKK4 and MKK7 kinase dead mutants are suitable substrates for phosphorylation by GST-MLK7.sub.KD and are expected to demonstrate a dose-dependent increase in signal strength with increasing substrate concentration. Subsequent experiments are routinely performed using 20 .mu.g/ml MKK4 as the substrate. Primary antibody 226.1 (New England BioLabs) dilution is assayed, and under conditions employed for the ELISA, a [fraction (1/5000)] dilution was determined to be optimal.

PGPUB-DOCUMENT-NUMBER: 20040181829

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040181829 A1

TITLE: Transgenic plants expressing a MAPKKK protein kinase domain

PUBLICATION-DATE: September 16, 2004

INVENTOR-INFORMATION:

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APPL-NO: 10/ 643434

DATE FILED: August 19, 2003

RELATED-US-APPL-DATA:

child 10643434 A1 20030819

parent continuation-of 09371338 19990810 US GRANTED

parent-patent 6613959 US

non-provisional-of-provisional 60095938 19980810 US

US-CL-CURRENT: 800/288, 435/194, 435/468

ABSTRACT:

The invention features plants including a recombinant transgene capable of expressing a kinase domain of a mitogen-activated protein kinase kinase kinase (MAPKKK) or a kinase domain thereof, wherein the transgene is expressed in said plant under the control of a promoter that is functional in a plant cell.

BACKGROUND OF THE INVENTION

[0001] This application is a continuation of 09/371,338 filed Aug. 10, 1999, and claims the benefit of U.S. provisional application Ser. No. 60/095,938 filed on Aug. 10, 1998.

---------- KWIC ---------

Detail Description Paragraph - DETX (22):

[0091] To further elucidate the molecular basis of oxidative stress signaling in plants, we have also showed that an Arabidopsis protoplast transient expression system is useful to investigate multiple stress responses. Three Arabidopsis stress responsive promoters, glutathione S-transferase GST6 (Chen et al., Plant J. 10: 995-966, 1996), heat shock HSP18.2 (Takahashi and Komeda, Mol. Gen. Genet. 219: 365-372, 1989), and the abscisic acid (ABA) responsive promoter RD29A (Yamaguchi-Shinozaki et al., Plant Physiol. 101: 1119-1120, 1993; Ishitani et al., Plant Cell 9: 1935-1949, 1997), were fused to

the luciferase (LUC) reporter and tested for their responses in transfected mesophyll protoplasts. The GST6, BSP18.2, and RD29A promoters were activated by H.sub.2O.sub.2, heat, and ABA, respectively, in protoplasts (FIG. 5A) as demonstrated previously in intact plants (Chen et al., supra; Takahashi and Komeda, supra; Yamaguchi-Shinozaki et al., supra; Ishitani et al., supra). Several GST genes, including GST6, have been shown to be induced by high and toxic concentrations of plant growth hormone auxin, as well as by physiologically inactive auxin analogs, heavy metals, and numerous stresses (Chen et al., supra; Ulmasov et al., Plant Mol. Biol. 26: 1055-1064, 1994; Abel and Theologis, Plant Physiol. 111: 9-17, 1996; Sitbon and Perrot-Rechenmann, Physiol Plantarum 100: 443-455, 1997; Guilfoyle et al., Plant Physiol., 118: 341-347, 1998, Marrs, Annu. Rev. Plant Physiol. Plant Mol. Biol. 47: 127-158, 1996). This non-specific induction of GSTs separates them from other auxin responsive genes that are only induced by low physiological levels of active auxin, and indicates that stress rather than auxin is responsible for the activation of the GST genes.

Detail Description Paragraph - DETX (35):

[0104] To determine whether a plant MAPKKK, such as ANP1 (Nishihama et al. Plant J. 12: 39-48, 1997), is involved in stress signal transduction, we have tested the effect of a constitutively active ANP1 kinase domain on the activity of several different dicot promoters. This was achieved by introducing into Arabidopsis protoplasts a transgene construct consisting of the firefly luciferase coding sequence (LUC) under the control of different dicot promoters. The promoters tested were the nitrate reductase, NR2, promoter from Arabidopsis (Lin et al., Plant Physiol. 106: 477-484, 1994); the asparagine synthetase, AS1, promoter (Neuhaus et al., EMBO J. 16: 2554-2564, 1997); the RD29A Arabidopsis stress-responsive promoter (Ishitani et al., Plant Cell 9: 1935-1949, 1997); the Arabidopsis HSP heat shock promoter (Sheen et al., Plant Journal 9: 777-784, 1995; Takahashi et al., Plant J. 2: 751-761, 1992); the Cab2 promoter (Mitra et al. Plant Mol. Biol. 12: 169-179, 1989); the chalcone synthase gene promoter (Feinbaum et al., Mol. Cell Biol. 8: 1985-1992, 1988); and the H.sub.2O.sub.2-inducible glutathione S-transferase promoter (GST) from Arabidopsis (Chen et al., Plant J. 10: 955-966, 1996). The kinase domain of ANP1 was cloned into a plant expression vector with a derivative of the 35S promoter and the nos terminator (Sheen, Science 274: 1900-1902, 1996). The ANP1 construct was co-transfected with one of the dicot promoter reporter gene construct and assayed according to standard methods. Surprisingly, the constitutively active ANP1 kinase domain was found to activate the expression of the AS1, HSP, and GST6 promoters (FIG. 7A). Constitutive expression of either the mutated NPK1 kinase domain or the CTR1 kinase domain had no effect on the expression of the dicot reporter genes.

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PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040110259 A1

TITLE: Drug metabolizing enzymes

PUBLICATION-DATE: June 10, 2004

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APPL-NO: 10/ 343593

DATE FILED: January 29, 2003

PCT-DATA:
APPL-NO: PCT/US01/24382
DATE-FILED: Aug 3, 2001
PUB-NO:
PUB-DATE:
371-DATE:
102(E)-DATE:

US-CL-CURRENT: 435/183, 435/252.3 , 435/320.1 , 435/325 , 435/69.1 , 536/23.2 , 800/8

ABSTRACT:

The invention provides human drug metabolizing enzymes (DME) and polynucleotides which identify and encode DME. The invention also provides expression vectors, host cells, antibodies, agonists, and antagonists. The invention also provides methods for diagnosing, treating, or preventing disorders associated with aberrant expression of DME.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (54):

[0051] In most cases, GSTs perform the beneficial finction of deactivation and detoxification of potentially mutagenic and carcinogenic chemicals. However, in some cases their action is detrimental and results in activation of chemicals with consequent mutagenic and carcinogenic effects. Some forms of rat and human GSTs are reliable preneoplastic markers that aid in the detection of carcinogenesis. Expression of human GSTs in bacterial strains, such as Salmonella tylhimurium used in the well-known Ames test for mutagenicity, has helped to establish the role of these enzymes in mutagenesis. Dihalomethanes, which produce liver tumors in mice, are believed to be activated by GST. This view is supported by the finding that dihalomethanes are more mutagenic in bacterial cells expressing human GST than in untransfected cells (Thier, R. et al.(1993) Proc. Natl. Acad. Sci. USA 90: 8567-80). The mutagenicity of ethylene dibromide and ethylene dichloride is increased in bacterial cells expressing the human Alpha GST, A1-1, while the mutagenicity of aflatoxin B1 is substantially reduced by enhancing the expression of GST (Simula, T. P. et al. (1993) Carcinogenesis 14:1371-6). Thus, control of GST activity maybe useful in the control of mutagenesis and carcinogenesis.

Detail Description Paragraph - DETX (85):

[0457] Protein arginine methyltransferase activity of DME is measured at 37.degree. C. for various periods of time. S-adenosyl-L-[methyl-.sup.3H]m-ethionine ([.sup.3H]AdoMet; specific activity=75 Ci/mmol; NEN Life Science Products) is used as the methyl-donor substrate. Useful methyl-accepting substrates include glutathione S-transferase fibrillarin glycine-arginine domain fusion protein (GST-GAR), heterogeneous nuclear rnbonucleoprotein (hnRNP), or hypomethylated proteins present in lysates from adenosine dialdehyde-treated cells. Methylation reactions are stopped by adding SDS-PAGE sample buffer. The products of the reactions are resolved by SDS-PAGE and visualized by fluorography. The presence, of .sup.3H-labeled methyl-donor substrates is indicative of protein argimne methyltransferase activity of DME (Tang, J. et al. (2000) J. Biol. Chem. 275:7723-7730and Tang, J. et al. (2000) J. BioL Chem.275:19866-19876).

PGPUB-DOCUMENT-NUMBER: 20040086887

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040086887 A1

TITLE: Drug metabolizing enzymes

PUBLICATION-DATE: May 6, 2004

INVENTOR-INFORMATION:

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APPL-NO: 10/ 381898

DATE FILED: March 27, 2003

PCT-DATA:
APPL-NO: PCT/US01/30662
DATE-FILED: Sep 28, 2001
PUB-NO:
PUB-DATE:
371-DATE:
102(E)-DATE:

US-CL-CURRENT: 435/6, 435/183 , 435/320.1 , 435/325 , 435/69.1 , 530/388.26 , 536/23.2

ABSTRACT:

The invention provides human drug metabolizing enzymes (DME) and polynucleotides which identify and encode DME. The invention also provides expression vectors, host cells, antibodies, agonists, and antagonists. The invention also provides methods for diagnosing, treating, or preventing disorders associated with aberrant expression of DME.

---------- KWIC --------

Summary of Invention Paragraph - BSTX (56):

[0053] In most cases, GSTs perform the beneficial function of deactivation and detoxification of potentially mutagenic and carcinogenic chemicals. However, in some cases their action is detrimental and results in activation of chemicals with consequent mutagenic and carcinogenic effects. Some forms of rat and human GSTs are reliable preneoplastic markers that aid in the detection of carcinogenesis. Expression of human GSTs in bacterial strains, such as Salmonella typhimurium used in the well-known Ames test for mutagenicity, has helped to establish the role of these enzymes in mutagenesis. Dihalomethanes, which produce liver tumors in mice, are believed to be activated by GST. This view is supported by the finding that dihalomethanes are more mutagenic in bacterial cells expressing human GST than in untransfected cells (Thier, R. et al. (1993) Proc. Natl. Acad. Sci. USA 90:8567-8580). The mutagenicity of ethylene dibromide and ethylene dichloride is increased in bacterial cells expressing the human Alpha GST, A1-1, while the mutagenicity of aflatoxin B1 is substantially reduced by enhancing the expression of GST (Simula, T. P. et al. (1993) Carcinogenesis 14:1371-1376). Thus, control of GST activity may be useful in the control of mutagenesis and carcinogenesis.

Detail Description Paragraph - DETX (88):

[0437] Protein arginine methyltransferase activity of DME is measured at 37.degree. C. for various periods of time. S-adenosyl-L[methyl-.sup.3H]methionine ([.sup.3H]AdoMet; specific activity=75 Ci/mmol; NEN Life Science Products) is used as the methyl-donor substrate. Useful methyl-accepting substrates include glutathione S-transferase fibrillarin glycine-arginine domain fusion protein (GST-GAR), heterogeneous nuclear ribonucleoprotein (hnRNP), or hypomethylated proteins present in lysates from adenosine dialdehyde-treated cells. Methylation reactions are stopped by adding SDS-PAGE sample buffer. The products of the reactions are resolved by SDS-PAGE and visualized by fluorography. The presence of .sup.3H-labeled methyl-donor substrates is indicative of protein arginine methyltransferase activity of DME (Tang, J. et al. (2000) J. Biol. Chem. 275:7723-7730 and Tang, J. et al. (2000) J. Biol. Chem.275:19866-19876).

PGPUB-DOCUMENT-NUMBER: 20040082061

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040082061 A1

TITLE: Drug metabolizing enzymes

PUBLICATION-DATE: April 29, 2004

INVENTOR-INFORMATION:

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APPL-NO: 10/ 468125

DATE FILED: August 15, 2003

PCT-DATA:
APPL-NO: PCT/US02/04918
DATE-FILED: Feb 14, 2002
PUB-NO:
PUB-DATE:
371-DATE:
102(E)-DATE:

US-CL-CURRENT: 435/320.1

ABSTRACT:

The invention provides human drug metabolizing enzymes (DME) and polynucleotides which identify and encode DME. The invention also provides expression vectors, host cells, antibodies, agonists, and antagonists. The invention also provides methods for diagnosing, treating, or preventing disorders associated with aberrant expression of DME.

---------- KWIC --------

Summary of Invention Paragraph - BSTX (58):

[0055] In most cases, GSTs perform the beneficial function of deactivation and detoxification of potentially mutagenic and carcinogenic chemicals. However, in some cases their action is detrimental and results in activation of chemicals with consequent mutagenic and carcinogenic effects. Some forms of rat and human GSTs are reliable preneoplastic markers that aid in the detection of carcinogenesis. Expression of human GSTs in bacterial strains, such as Salmonella typhimurium used in the well-known Ames test for mutagenicity, has helped to establish the role of these enzymes in mutagenesis. Dihalomethanes, which produce liver tumors in mice, are believed to be activated by GST. This view is supported by the finding that dihalomethanes are more mutagenic in bacterial cells expressing human GST than in untransfected cells (Thier, R. et al. (1993) Proc. Natl. Acad. Sci. USA 90:8567-8580). The mutagenicity of ethylene dibromide and ethylene dichloride is increased in bacterial cells expressing the human Alpha GST, A1-1, while the mutagenicity of aflatoxin B1 is substantially reduced by enhancing the expression of GST (Simula, T. P. et al. (1993) Carcinogenesis 14:1371-1376). Thus, control of GST activity may be useful in the control of mutagenesis and carcinogenesis.

Detail Description Paragraph - DETX (91):

[0487] Protein arginine methyltransferase activity of DME is measured at 37.degree. C. for various periods of time. S-adenosyl-L-[methyl-3H]methionine ([.sup.3H]AdoMet; specific activity=75 Ci/mmol; NEN Life Science Products) is used as the methyl-donor substrate. Useful methyl-accepting substrates include glutathione S-transferase fibrillarin glycine-arginine domain fusion protein (GST-GAR), heterogeneous nuclear ribonucleoprotein (hnRNP), or hypomethylated proteins present in lysates from adenosine dialdehyde-treated cells. Methylation reactions are stopped by adding SDS-PAGE sample buffer. The products of the reactions are resolved by SDS-PAGE and visualized by fluorography. The presence of .sup.3H-labeled methyl-donor substrates is indicative of protein arginine methyltransferase activity of DME (Tang, J. et al. (2000) J. Biol. Chem. 275:7723-7730 and Tang, J. et al. (2000) J. Biol. Chem.275: 19866-19876).

PGPUB-DOCUMENT-NUMBER: 20040081980

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040081980 A1

TITLE: Drug metabolizing enzymes

PUBLICATION-DATE: April 29, 2004


INVENTOR-INFORMATION:

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| Yang, Junming | San Jose | CA | US | |
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APPL-NO: 10/ 433256

DATE FILED: May 30, 2003

PCT-DATA:
APPL-NO: PCT/US01/47429
DATE-FILED: Dec 4, 2001
PUB-NO:
PUB-DATE:
371-DATE:
102(E)-DATE:

US-CL-CURRENT: 435/6, 435/183 , 435/320.1 , 435/325 , 435/69.1 , 530/388.26 , 536/23.2



ABSTRACT:

The invention provides human drug metabolizing enzymes (DME) and polynucleotides which identify and encode DME. The invention also provides expression vectors, host cells, antibodies, agonists, and antagonists. The invention also provides methods for diagnosing, treating, or preventing disorders associated with aberrant expression of DME.

---------- KWIC --------

Summary of Invention Paragraph - BSTX (57):

[0054] In most cases, GSTs perform the beneficial function of deactivation and detoxification of potentially mutagenic and carcinogenic chemicals. However, in some cases their action is detrimental and results in activation of chemicals with consequent mutagenic and carcinogenic effects. Some forms of rat and human GSTs are reliable preneoplastic markers that aid in the detection of carcinogenesis. Expression of human GSTs in bacterial strains, such as Salmonella typhimurium used in the well-known Ames test for mutagenicity, has helped to establish the role of these enzymes in mutagenesis. Dihalomethanes, which produce liver tumors in mice, are believed to be activated by GST. This view is supported by the finding that dihalomethanes are more mutagenic in bacterial cells expressing human GST than in untransfected cells (Thier, R. et al. (1993) Proc. Natl. Acad. Sci. USA 90:8567-8580). The mutagenicity of ethylene dibromide and ethylene dichloride is increased in bacterial cells expressing the human Alpha GST, A1-1, while the mutagenicity of aflatoxin B1 is substantially reduced by enhancing the expression of GST (Simula, T. P. et al. (1993) Carcinogenesis 14:1371-1376). Thus, control of GST activity may be useful in the control of mutagenesis and carcinogenesis.

Detail Description Paragraph - DETX (84):

[0462] Protein arginine methyltransferase activity of DME is measured at 37.degree. C. for various periods of time. S-adenosyl-L-[methyl-.sup.3H]m-ethionine ([.sup.3H]AdoMet; specific activity=75 Ci/mmol; NEN Life Science Products) is used as the methyl-donor substrate. Useful methyl-accepting substrates include glutathione S-transferase fibrillarin glycine-arginine domain fusion protein (GST-GAR), heterogeneous nuclear ribonucleoprotein (hnRNP), or hypomethylated proteins present in lysates from adenosine dialdehyde-treated cells. Methylation reactions are stopped by adding SDS-PAGE sample buffer. The products of the reactions are resolved by SDS-PAGE and visualized by fluorography. The presence of .sup.3H-labeled methyl-donor substrates is indicative of protein arginine methyltransferase activity of DME (Tang, J. et al. (2000) J. Biol. Chem. 275:7723-7730 and Tang, J. et al. (2000) J. Biol. Chem. 275:19866-19876).

Detail Description Table CWU - DETL (2):

4TABLE 2 Incyte Polypeptide Polypeptide GenBank ID Probability SEQ ID NO: ID NO: score GenBank Homolog 1 7483477CD1 g203890 6.9e-225 Cytochrome P-450 [Rattus norvegicus]. Nef, P. et al. (1990) Olfactory-specific cytochrome P- 450 (P-450olf1; IIG1): Gene structure and developmental regulation. J. Biol. Chem. 265: 2903-2907. 2 7485159CD1 g8132762 1.6e-83 Glutathione transferase omega [Homo sapiens]. Board, P. G. et al. (2000) Identification, characterization, and crystal structure of the Omega class glutathione transferases. J. Biol. Chem. 275: 24798-24806. 3 7485518CD1 g4322247 0.0 Heparan sulfate N-deacetylase/N-sulfotransfe- rase 3. [Homo sapiens]. Aikawa, J. I. et al. (1999) Molecular cloning and expression of a third member of the heparan sulfate/heparin GlcNAc N-deacetylase/N-sulfotransferase family. J. Biol. Chem. 274: 2690-2695. 4 2860635CD1 g2121220 8.7e-172 Polypeptide GalNAc transferase-T4 [Mus musculus]. 5 2530615CD1 g1145789 0.0 Neuroligin 2 [Rattus norvegicus]. Ichtchenko, K. et al. (1996) Structures, alternative splicing, and neurexin binding of multiple neuroligins. J. Biol. Chem. 271: 2676-2682. 6 3883906CD1 g971461 3.0e-146 UDP-GalNAc: polypeptide N-acetylgalactosaminyl transferase [Homo sapiens]. White, T. et al. (1995) Purification and cDNA cloning of a human UDP-N-acetyl-alpha-D- galactosamine: polypeptide N-acetylgalactosaminyltransferase. J. Biol. Chem. 270: 24156-24165. 7 7473644CD1 g1049108 1.5e-128 Carbonyl reductase [Mus musculus]. Wei, J.

(1996) Cloning a cDNA for carbonyl reductase (Cbr) from mouse cerebellum: murine genes that express cbr map to chromosomes 16 and 11. Genomics 34: 147-148. 8 7485303CD1 g1698601 1.1e-169 Beta-1,6-N-acetylglucosaminyltransferase [Cricetulus griseus]. Weinstein, J. et al. (1996) A point mutation causes mistargeting of Golgi GlcNAc-TV in the Lec4A Chinese hamster ovary glycosylation mutant. J. Biol. Chem. 271: 27462-27469. 9 6970969CD1 g9946340 5.8e-56 Probable N-acetyltransferase [Pseudomonas aeruginosa]. Stover, C. K. et al. (2000) Complete genome sequence of Pseudomonas aeruginosa PA01, an opportunistic pathogen. Nature 406: 959-964. 10 6538080CD1 g2641986 1.9e-130 Carboxylesterase precursor [Mesocricetus auratus]. 11 55048919CD1 g144283 4.1e-32 Cu/Zn-superoxide dismutase [Caulobacter crescentus]. Steinman, H. M. and Ely, B. (1990) Copper-zinc superoxide dismutase of Caulobacter crescentus; Cloning, sequencing, and mapping of the gene and periplasmic location of the enzyme. J. Bacteriol. 172: 2901-2910. 12 7485135CD1 g306810 6.3e-98 Glutathione S-transferase Ha subunit 1 (EC 2.5.1.18) [Homo sapiens]. Rhoads, D. M. et al. (1987) The basic glutathione S- transferases from human livers are products of separate genes. Biochem. Biophys. Res. Commun. 145: 474-481. 13 7684978CD1 g1736409 8.0e-75 Morphine 6-dehydrogenase (EC 1.1.1.218) (Naloxone reductase) [Escherichia coli]. Itoh, T. et al. (1996) A 460-kb DNA sequence of the Escherichia coli K-12 genome corresponding to the 40.1-50.0 min region on the linkage map. DNA Res. 3: 379-392.

PGPUB-DOCUMENT-NUMBER: 20040072159

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040072159 A1

TITLE: Bzip type transcription factors regulating the expression of rice storage protein

PUBLICATION-DATE: April 15, 2004

INVENTOR-INFORMATION:

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APPL-NO: 10/ 149553

DATE FILED: December 19, 2002

FOREIGN-APPL-PRIORITY-DATA:

| COUNTRY | APPL-NO | DOC-ID | APPL-DATE |
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| JP | 2000-311295 | 2000JP-2000-311295 | October 11, 2000 |

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APPL-NO: PCT/JP01/08936
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US-CL-CURRENT: 435/6, 435/199 , 435/320.1 , 435/419 , 435/69.1 , 536/23.2

ABSTRACT:

cDNAs (RISBZ1, RISBZ4, and RISBZ5) encoding bZIP transcription factors were isolated from a cDNA library originating in rice plant seed. The cDNAs encode novel proteins and have binding activity to the GCN4 motif. Among them, RISBZ1 activated transcription mediated by the GCN4 motif by 100-fold or more. Since the expression of RISBZ1 precedes the expression of a seed storage protein gene and is expressed only in maturing seeds, it is suggested that RISBZ1 controls the expression of rice seed storage proteins. In addition, by linking the recognition sequence of the transcription factor, the GCN4 motif, in tandem and introducing it into the promoter for a gene encoding seed storage protein to facilitate its binding to the transcription factor RISBZ1, expression of a foreign gene under the control of the modified promoters is greatly enhanced.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (8):

[0006] Opaque2 (O2) of maize is an endosperm-specific transcription factor of the bZIP type, and this O2 binds to the ACGT motif in the 22 kDa .alpha.-zein gene promoter of maize to activate transcription (Schmidt, R. J. et al., Plant Cell 4: 689-700, 1992). O2 has been reported to be involved in endosperm-specific transcription of b-32 ribosome deactivating protein gene by

binding to the (Ga/tTGAPyPuTGPu) sequence (Lohmer, S. et al., EMBO J. 10: 617-624, 1991). O2 is thus considered to have a wide-ranging binding capability. Reportedly, the GCN4 motif is recognized by O2, and transcription is activated through the binding of O2 to the GCN4 motif (Wu, C. Y. et al., Plant J. 14: 673-683, 1998; Holdsworth, M. J. et al., Plant Mol. Biol. 29: 711-720, 1995). In seeds, during the maturing stage, in vivo footprint analysis showed that the nuclear protein binds to the GCN4 motif and Prolamin box present in wheat low molecular weight glutenin gene promoter (Vicente-Carbajos, J. et al., Plant J. 13: 629-640, 1998) and maize .gamma.-zein gene promoter (Marzabal, P. M. et al., Plant J. 16: 41-52, 1998). In addition, the results of an in vitro DNaseI footprint analysis showed that the nuclear protein of maturing rice plant seeds as well as GST-02 fused protein specifically recognize the GCN4 motif of the rice glutelin gene promoter (Wu, C. Y., et al., Plant J. 14: 673-683, 1998; Kim, S. Y. and Wu, R., Nucl. Acids Res. 18: 6845-6852, 1990). These findings indicate that an O2-like transcription factor is present in grain seeds, and that it controls the endosperm-specific expression of numerous seed storage protein genes mediated by the GCN4 motif.

Detail Description Paragraph - DETX (54):

[0140] A pair of oligonucleotides complementary to each other, which was prepared by adding TCGA sequence was added to 21-nt fragment of GluB1 promoter region (from -175.sup.th to -155.sup.th), was labeled at its ends with [.alpha.-.sup.32P] dCTP by `fill-in` reaction for use as a probe. Seven pairs of complementary oligonucleotides with mutations every three contiguous nucleotides (FIG. 8A) were also synthesized for use as mutant competitor fragments and were annealed. Gel shift analysis using the GST fusion protein was carried out by a method described by Wu et al. (Wu C. Y. et al. Plant J. 14: 673-683, 1998) and by Suzuki et al. (Suzuki A. et al. Plant Cell Physiol. 39: 555-559, 1998). The labeled oligonucleotide probe was mixed with 0.5 .mu.g of the GST-RISBZ fusion protein, and incubated for 20 min at room temperature. In competition experiments, the competitor fragment was added to the mixture at the 100-fold or higher molecular weight ratio. The reacted mixture was analyzed by non-denaturing acrylamide gel (5%, 0.25.times.TBE) electrophoresis.

Detail Description Paragraph - DETX (55):

[0141] The detection of shift bands showed that the GST-RISBZ1 protein was able to bind to the 21-bp DNA fragment containing the GCN4 motif (FIG. 8B). Furthermore, as shown FIG. 8A, the 21-bp DNA fragments with mutation in every three contiguous nucleotides were used as competitors and examined. When the DNA fragments with the mutations in the GCF motif were added as the competitor, the binding of the DNA fragments that were added as probes was hardly or not inhibited at all (FIGS. 8B to F). By contrast, when the DNA fragments with mutations in the franking sequence of the GCN4 motif were added as the competitor, the shift bands disappeared (FIGS. 8B to F). Since the mutation of the GCN4 motif markedly affects the binding of the RISBZ1 protein to the motif, it was revealed that the RISBZ1 protein recognizes the GCN4 motif sequence specifically. The similar experiments carried out using the other RISBZ proteins revealed that all the RISBZ proteins could specifically recognize the GCN4 motif. As shown in FIGS. 8B to F, the affinity of each RISBZ proteins for the GCN4 motif slightly varies. In the cases of RISBZ2 and RISBZ5, when the DNA fragments with mutations in the franking sequence of the GCN motif were used as the competitor, the shift bands were not disappeared completely (FIGS. 8C and F).

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PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040029132 A1

TITLE: Drug metabolizing enzymes

PUBLICATION-DATE: February 12, 2004

INVENTOR-INFORMATION:

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APPL-NO: 10/ 296606

DATE FILED: May 2, 2003

PCT-DATA:
APPL-NO: PCT/US01/17150
DATE-FILED: May 25, 2001
PUB-NO:
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102(E)-DATE:

US-CL-CURRENT: 435/6, 435/183 , 435/320.1 , 435/325 , 435/69.1 , 530/388.26 , 536/23.2

ABSTRACT:

The invention provides human drug metabolizing enzymes (DME) and polynucleotides which identify and encode DME. The invention also provides expression vectors, host cells, antibodies, agonists, and antagonists. The

invention also provides methods for diagnosing, treating or preventing disorders associated with aberrant expression of DME.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (56):

[0053] In most cases, GSTs perform the beneficial function of deactivation and detoxification of potentially mutagenic and carcinogenic chemicals. However, in some cases their action is detrimental and results in activation of chemicals with consequent mutagenic and carcinogenic effects. Some forms of rat and human GSTs are reliable preneoplastic markers that aid in the dection of carcinogenesis. Expression of human GSTs in bacterial strains, such as Salmonella typhimurium used in the well-known Ames test for mutagenicity, has helped to establish the role of these enzymes in mutagenesis. Dihalomethanes, which produce liver tumors in mice, are believed to be activated by GST. This view is supported by the finding that dihalomethanes are more mutagenic in bacterial cells expressing human GST than in untransfected cells (Thier, R. et al. (1993) Proc. Natl. Acad. Sci. USA 90:8567-8580). The mutagenicity of ethylene dibromide and ethylene dichloride is increased in bacterial cells expressing the human Alpha GST, AI -1, while the mutagenicity of aflatoxin B 1 is substantially reduced by enhancing the expression of GST (Simula, T. P. et al. (1993) Carcinogenesis 14:1371-1376). Thus, control of GST activity may be useful in the control of mutagenesis and carcinogenesis.

Detail Description Paragraph - DETX (84):

[0440] Protein arginine methyltransferase activity of DME is measured at 37.degree. C. for various periods of time. S-adenosyl-L-[methyl-3H]methionine ([3H]AdoMet; specific activity=75 Ci/mmol; NEN Life Science Products) is used as the methyl-donor substrate. Useful methyl-accepting substrates include glutathione S-transferase fibrillarin glycine-arginine domain fusion protein (GST-GAR), heterogeneous nuclear ribonucleoprotein (hnRNP), or hypomethylated proteins present in lysates from adenosine dialdehyde-treated cells. Methylation reactions are stopped by adding SDS-PAGE sample buffer. The products of the reactions are resolved by SDS-PAGE and visualized by fluorography. The presence of .sup.3H-labeled methyl-donor substrates is indicative of protein arginine methyltransferase activity of DME (Tang, J. et al. (2000) J. Biol. Chem. 275:7723-7730 and Tang, J. et al. (2000) J. Biol. Chem. 275:19866-19876).

PGPUB-DOCUMENT-NUMBER: 20040029125

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040029125 A1

TITLE: Drug metabolizing enzymes

PUBLICATION-DATE: February 12, 2004

INVENTOR-INFORMATION:

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DATE FILED: October 15, 2002

PCT-DATA:
APPL-NO: PCT/US01/11869
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371-DATE:
102(E)-DATE:

US-CL-CURRENT: 435/6, 435/183 , 435/320.1 , 435/325 , 435/69.1 , 536/23.2 , 800/8

ABSTRACT:

The invention provides human drug metabolizing enzymes (DME) and polynucleotides which identify and encode DME. The invention also provides expression vectors, host cells, antibodies, agonists, and antagonists. The invention also provides methods for diagnosing, treating, or preventing disorders associated with aberrant expression of DME.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (56):

[0053] In most cases, GSTs perform the beneficial function of deactivation and detoxification of potentially mutagenic and carcinogenic chemicals. However, in some cases their action is detrimental and results in activation of chemicals with consequent mutagenic and carcinogenic effects. Some forms of rat and human GSTs are reliable preneoplastic markers that aid in the detection of carcinogenesis. Expression of human GSTs in bacterial strains, such as Salmonella typhimurium used in the well-known Ames test for mutagenicity, has helped to establish the role of these enzymes in mutagenesis. Dihalomethanes, which produce liver tumors in mice, are believed to be activated by GST. This view is supported by the finding that dihalomethanes are more mutagenic in bacterial cells expressing human GST than in untransfected cells (Thier, R. et al. (1993) Proc. Natl. Acad. Sci. USA 90:8567-8580). The mutagenicity of ethylene dibromide and ethylene dichloride is increased in bacterial cells expressing the human Alpha GST, A1-1, while the mutagenicity of aflatoxin B1 is substantially reduced by enhancing the expression of GST (Simula, T. P. et al. (1993) Carcinogenesis 14:1371-1376). Thus, control of GST activity may be useful in the control of mutagenesis and carcinogenesis.

Detail Description Paragraph - DETX (86):

[0445] Protein arginine methyltransferase activity of DME is measured at 37.degree. C. for various periods of time. S-adenosyl-L-[methyl-.sup.3H]m-ethionine ([.sup.3H]AdoMet; specific activity=75 Ci/mmol; NEN Life Science Products) is used as the methyl-donor substrate. Useful methyl-accepting substrates include glutathione S-transferase fibrillarin glycine-arginine domain fusion protein (GST-GAR), heterogeneous nuclear ribonucleoprotein (hnRNP), or hypomethylated proteins present in lysates from adenosine dialdehyde-treated cells. Methylation reactions are stopped by adding SDS-PAGE sample buffer. The products of the reactions are resolved by SDS-PAGE and visualized by fluorography. The presence of .sup.3H-labeled methyl-donor substrates is indicative of protein arginine methyltransferase activity of DME (Tang, J. et al. (2000) J. Biol. Chem. 275:7723-7730 and Tang, J. et al. (2000) J. Biol. Chem. 275:19866-19876).

PGPUB-DOCUMENT-NUMBER: 20030219763

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030219763 A1

TITLE: Plant protoplast gene expression systems and uses thereof

PUBLICATION-DATE: November 27, 2003

INVENTOR-INFORMATION:

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APPL-NO: 10/ 243581

DATE FILED: September 13, 2002

RELATED-US-APPL-DATA:

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parent continuation-of PCT/US01/07999 20010313 US PENDING

child 10243581 A1 20020913

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child 10243581 A1 20020913

parent continuation-in-part-of 09371338 19990810 US GRANTED

parent-patent 6613959 US

non-provisional-of-provisional 60189074 20000313 US

non-provisional-of-provisional 60032966 19961213 US

non-provisional-of-provisional 60095938 19980810 US

US-CL-CURRENT: 435/6

ABSTRACT:

Disclosed are high throughput assays for rapidly screening a library of nucleic acid molecules to identify a gene product that modulates expression of a gene of interest. The assays generally involve (a) introducing into one or more plant protoplasts (i) a reporter gene construct operably linked to a promoter of a gene of interest and (ii) a member of a library of nucleic acid molecules, wherein the library member is expressed in the plant protoplasts; and (b) screening the protoplasts to determine whether the amount of gene expression of the reporter gene construct changes in response to the expression of the library member, a change in gene expression of the reporter gene construct identifying the gene product expressed by the library member as one that modulates expression of the gene of interest.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit of PCT international application number US01/07999, filed on Mar. 13, 2001, which, in turn, claims benefit of U.S. provisional patent application No. 60/189,074, filed on Mar. 13, 2000, now abandoned, and is a continuation-in-part of U.S. patent application Ser. No. 08/989,881, filed on Dec. 12, 1997, which claims benefit of U.S. provisional application No. 60/032,966, filed on Dec. 13, 1996, now abandoned, and U.S. patent application Ser. No. 09/371,338, filed on Aug. 10, 1999, which claims benefit of U.S. provisional application No. 60/095,938, filed on Aug. 10, 1998, now abandoned.

---------- KWIC ---------

Detail Description Paragraph - DETX (45):

[0116] To further elucidate the molecular basis of oxidative stress signaling in plants, we have also showed that an Arabidopsis protoplast transient expression system is useful to investigate multiple stress responses. Three Arabidopsis stress responsive promoters, glutathione S-transferase GST6 (Chen et al., Plant J. 10: 995-966, 1996), heat shock HSP18.2 (Takahashi and Komeda, Mol. Gen. Genet. 219: 365-372, 1989), and the abscisic acid (ABA) responsive promoter RD29A (Yamaguchi-Shinozaki et al., Plant Physiol. 101: 1119-1120, 1993; Ishitani et al., Plant Cell 9: 1935-1949, 1997), were fused to the luciferase (LUC) reporter and tested for their responses in transfected mesophyll protoplasts. The GST6, HSP18.2, and RD29A promoters were activated by H.sub.2O.sub.2, heat, and ABA, respectively, in protoplasts (FIG. 10A) as demonstrated previously in intact plants (Chen et al., supra; Takahashi and Komeda, supra; Yamaguchi-Shinozaki et al., supra; Ishitani et al., supra). Several GST genes, including GST6, have been shown to be induced by high and toxic concentrations of plant growth hormone auxin, as well as by physiologically inactive auxin analogs, heavy metals, and numerous stresses (Chen et al., supra; Ulmasov et al., Plant Mol. Biol. 26: 1055-1064, 1994; Abel and Theologis, Plant Physiol. 111: 9-17, 1996; Sitbon and Perrot-Rechenmann, Physiol. Plantarum 100: 443-455, 1997; Guilfoyle et al., Plant Physiol., 118: 341-347, 1998, Marrs, Annu. Rev. Plant Physiol. Plant Mol. Biol. 47: 127-158, 1996). This non-specific induction of GSTs separates them from other auxin responsive genes that are only induced by low physiological levels of active auxin, and indicates that stress rather than auxin is responsible for the activation of the GST genes.

Detail Description Paragraph - DETX (61):

[0132] To determine whether a plant MAPKKK, such as ANP1 (Nishihama et al. Plant J. 12: 39-48, 1997), is involved in stress signal transduction, we have tested the effect of a constitutively active ANP1 kinase domain on the activity of several different dicot promoters. This was achieved by introducing into Arabidopsis protoplasts a transgene construct consisting of the firefly luciferase coding sequence (LUC) under the control of different dicot promoters. The promoters tested were the nitrate reductase, NR2, promoter from Arabidopsis (Lin et al., Plant Physiol. 106: 477-484, 1994); the asparagine synthetase, AS1, promoter (Neuhaus et al., EMBO J. 16: 2554-2564, 1997); the RD29A Arabidopsis stress-responsive promoter (Ishitani et al., Plant Cell 9: 1935-1949, 1997); the Arabidopsis HSP heat shock promoter (Sheen et al., Plant Journal 9: 777-784, 1995; Takahashi et al., Plant J. 2: 751-761, 1992); the Cab2 promoter (Mitra et al. Plant Mol. Biol. 12: 169-179, 1989); the chalcone synthase gene promoter (Feinbaum et al., Mol. Cell Biol. 8: 1985-1992, 1988); and the H.sub.2O.sub.2-inducible glutathione S-transferase promoter (GST) from Arabidopsis (Chen et al., Plant J. 10: 955-966, 1996). The kinase domain of ANP1 was cloned into a plant expression vector with a derivative of the 35S promoter and the nos terminator (Sheen, Science 274: 1900-1902, 1996). The

ANP1 construct was co-transfected with one of the dicot promoter reporter gene construct and assayed according to standard methods. Surprisingly, the constitutively active ANP1 kinase domain was found to activate the expression of the AS1, HSP, and GST6 promoters (FIG. 12A). Constitutive expression of either the mutated NPK1 kinase domain or the CTR1 kinase domain had no effect on the expression of the dicot reporter genes.

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PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030167516 A1

TITLE: Calcium dependent protein kinase polypeptides as regulators of plant disease resistance

PUBLICATION-DATE: September 4, 2003

INVENTOR-INFORMATION:

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APPL-NO: 09/ 848806

DATE FILED: May 4, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60201925 20000505 US

US-CL-CURRENT: 800/279

ABSTRACT:

In general, the invention features a method of producing a plant having increased disease resistance. The method includes the steps of: (a) providing a non-naturally occurring plant cell overexpressing a nucleic acid molecule encoding a calcium dependent protein kinase (CDPK) polypeptide; and (b) regenerating a plant from said plant cell, wherein the CDPK polypeptide is expressed in said plant, increasing the resistance of the plant to disease as compared to a naturally-occurring plant. In addition, the invention In related aspects, the invention further features non-naturally occurring plants (or plant cell, plant tissue, plant organ, or plant component) that expresses a nucleic acid molecule encoding either a CDPK2 or CDPK4 polypeptides or both, as well as seeds and cells of such plants.

[0001] This application claims benefit of U.S. provisional patent application 60/201,925, filed on May 5, 2000, which is hereby incorporated by reference in its entirety.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (38):

[0037] Furthermore, because the invention reduces the necessity for chemical protection against plant pathogens, the invention benefits the environment where the crops are grown. Genetically-improved seeds and other plant products that are produced using plants expressing the genes described herein also render farming possible in areas previously unsuitable for agricultural production. The invention further provides a means for mediating the expression of early pathogen responsive genes, for example, phenylalanine ammonia-lyase (PAL) and glutathione S-transferase (GST), that confer resistance to plant pathogens. For example, transgenic plants constitutively producing a

constitutively-active CDPK gene product are capable of activating gene expression, which, in turn, confers resistance to plant pathogens. Collective activation of early pathogen response gene expression that is mediated by the CDPK gene product obviates the need to express these genes individually as a means to promote plant defense mechanisms.

Detail Description Paragraph - DETX (6):

[0047] To determine whether CDPK2 (Urao et al., Mol. Gen. Genet. 244: 331-340, 1994) is involved in disease resistance, I tested the effect of a constitutively active CDPK2 polypeptide on the activity of several inducible promoters activated in early pathogen defense. The promoters tested were the chalcone synthase ("CHS") promoter (Feinbaum et al., Mol. Cell Biol. 8: 1985-1992, 1988); the phenylalanine lyase ("PAL1") promoter (Ohl et al., Plant Cell 2: 837-848, 1990); the ethylene enhancer element ("GCC1") (Fujimoto et al., Plant Cell 12: 393-404, 2000); and the glutathione S-transferase promoter ("GST1") (Yang et al., Plant Cell Rep. 17: 700-704, 1999). Chimeric genes were generated by fusing the inducible promoters to a firefly luciferase sequence (Sheen, Science 274: 1900-1902, 1996; Kotvun et al., Proc. Natl. Acad. Sci. 97: 2940-2945, 2000). The CDPK2 polypeptide including the protein kinase domain was cloned into a plant expression vector with a derivative of the 35S promoter and the nos terminator (Sheen, Science 274: 1900-1902, 1996) using standard methods. Arabidopsis protoplasts were co-transfected with one of the dicot promoter firefly luciferase reporter gene constructs and the CDPK2 construct, and assayed according to the standard methods (Sh, Science 274: 1900-1902, 1996; Kotvun et al., Proc. Natl. Acad. Sci. 97: 2940-5,2000). The constitutively active CDPK2 polypeptide was found to activate the expression of the CHS, PAL1, GCC1, and GST promoters. Constitutive expression of a mutated CDPK2 polypeptide, APK1, or the serine-threonine protein kinase, ASK1 (Sheen, Science.274: 1900-1902, 1996) had no effect on the expression of the dicot reporter genes. Similar results were found when constitutively-active CDPK4 was used.

PGPUB-DOCUMENT-NUMBER: 20030074699

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030074699 A1

TITLE: Genetic control of flowering

PUBLICATION-DATE: April 17, 2003

INVENTOR-INFORMATION:

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APPL-NO: 10/ 143849

DATE FILED: May 14, 2002

RELATED-US-APPL-DATA:

child 10143849 A1 20020514

parent continuation-of 09516191 20000301 US ABANDONED

child 09516191 20000301 US

parent continuation-of 08945056 19971020 US GRANTED

parent-patent 6077994 US

child 08945056 19971020 US

parent a-371-of-international PCT/GB95/02561 19951101 WO UNKNOWN

FOREIGN-APPL-PRIORITY-DATA:

| COUNTRY | APPL-NO | DOC-ID | APPL-DATE |
|---|---|---|---|
| GB | 9422083.7 | 1994GB-9422083.7 | November 2, 1994 |

US-CL-CURRENT: 800/287, 435/183 , 435/320.1 , 435/419 , 536/23.6

ABSTRACT:

The CONSTANS (CO) gene of Arabidopsis thalina and homologues from Brassica napus are provided and are useful for influencing flowering characteristics in transgenic plants, especially the timing of flowering.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (42):

[0042] As stated above, the expression pattern of the CO gene may be altered by fusing it to a foreign promoter. For example, International patent application WO93/01294 of Imperial Chemical Industries Limited described a chemically inducible gene promoter sequence isolated from a 27 kD subunit of the maize glutathione-S-transferase, isoform II gene (GST-II-27) (see FIG. 2). It has been found that when linked to an exogenous gene and introduced into a plant by transformation, the GST-II-27 promoter provides a means for the

external regulation of the expression of that exogenous gene. The structural region of the CO gene is fused to the GST-II-27 promoter downstream of the translation start point shown in FIG. 2.

Summary of Invention Paragraph - BSTX (44):

[0044] Accordingly, the present invention provides in a further aspect a gene construct comprising an inducible promoter operatively linked to a nucleotide sequence provided by the present invention, such as the CO gene of Arabidopsis thaliana, a homologue from another plant species or any mutant, derivative or allele thereof. This enables control of expression of the gene. The invention also provides plants transformed with said gene construct and methods comprising introduction of such a construct into a plant cell and/or induction of expression or a construct within a plant cell, by application of a suitable stimulus, an effective exogenous inducer. The promoter may be the GST-II-27 gene promoter or any other inducible plant promoter. Promotion of CO activity to cause early flowering

PGPUB-DOCUMENT-NUMBER: 20030041357

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030041357 A1

TITLE: Herbicide resistant plants

PUBLICATION-DATE: February 27, 2003

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
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APPL-NO: 09/ 791489

DATE FILED: February 23, 2001

RELATED-US-APPL-DATA:

child 09791489 A1 20010223

parent division-of 09297706 19990505 US ABANDONED

child 09297706 19990505 US

parent a-371-of-international PCT/GB97/02996 19971031 WO UNKNOWN

FOREIGN-APPL-PRIORITY-DATA:

| COUNTRY | APPL-NO | DOC-ID | APPL-DATE |
|---|---|---|---|
| GB | 9623248.3 | 1996GB-9623248.3 | November 7, 1996 |
| GB | 9625957.7 | 1996GB-9625957.7 | December 13, 1996 |
| GB | 9703855.8 | 1997GB-9703855.8 | February 25, 1997 |

US-CL-CURRENT: 800/300, 435/320.1 , 536/23.7 , 800/278

ABSTRACT:

The present invention provides inter alia, a polynucleotide comprising at least a first region encoding a first protein capable of conferring on a plant, or tissue comprising it, resistance or tolerance to a first herbicide, and a second region encoding a second protein likewise capable of conferring resistance to a second herbicide, with the provisos (i) that the polynucleotide does not encode a fusion protein comprising only a 5-enol-pyruvyl-3-phosphoshikimate synthetase (EPSPS) and a glutathione S transferase (GST); (ii) that the polynucleotide does not comprise only regions encoding superoxide dismutase (SOD) and glutathione S transferase (GST), and (iii) that the polynucleotide does not comprise only regions encoding GST and phosphinothricin acetyl transferase (PAT).

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (12):

[0012] The proteins encoded by the said regions of the polynucleotide may be selected from the group consisting of glyphosate oxido-reductase (GOX), 5-enol-pyruvyl-3-phosphoshikimate synthetase (EPSPS), phosphinothricin acetyl transferase (PAT), hydroxyphenyl pyruvate dioxygenase (HPPD), glutathione S transferase (GST), cytochrome P450. Acetyl-COA carboxylase (ACC), Acetolactate synthase (ALS), protoporphyrinogen oxidase (protox), dihydropteroate synthase, polyamine transport proteins, superoxide dismutase (SOD), bromoxynil nitrilase (BNX), phytoene desaturase (PDS), the product of the tfdA gene obtainable from Alcaligenes eutrophus, and mutagenised or otherwise modified variants of the said proteins. The product of the said tfdA gene is a dioxygenase which is capable of oxidising phenoxycarboxylic acids, such as 2,4-D to the corresponding phenol. The EPSPS enzyme may be a so called class II EPSPS, as described in European Patent No. 546,090. Alternatively, and/or additionally, it may be mutated so as to comprise amino acid substitutions at certain positions which are known to result in enhanced resistance to glyphosate (and agriculturally acceptable salts thereof). For example, the EPSPS may have at least the residues Thr, Pro, Gly and Ala at positions corresponding to 174, 178, 173 and 264 with respect to the EPSPS depicted in SEQ ID No. 9 alerted as follows:

Detail Description Paragraph - DETX (42):
Cloning of the GST Gene into Plant Material and the Generation of Plants Resistant to Anilide and Diphenyl Ether Type Herbicides

Detail Description Paragraph - DETX (100):
[0144] The calli which contain the GST-27 expression cassette are transferred to plant regeneration media and maize plants are recovered. The transformed maize plants are confirmed--by Western blots of total protein extracts from leaves--to constitutively express the GST gene at high levels. Such plants are cross pollinated with an elite maize inbred line and seed is recovered. To confirm enhanced tolerance of the plants to the herbicide acetochlor the said seeds are planted in soil to which has been applied between 2,000 and 8,000 grams per hectare of the herbicide. The seeds are allowed to germinate and grow for 7 days after which time a sample of the resultant seedlings is assessed for damage caused the chemical and compared to the seedlings (if any) which result from non-transgenic seed sown under identical conditions. The "transgenic" seedlings and non-transgenic control seedlings grown in soil treated with the herbicide and a corresponding safener exhibit little, if any damage, whereas non-transgenic seedlings grown in soil which contains herbicide in the absence of safener show very substantial damage. Seedlings which survive the first herbicide treatment are allowed to grow for a farther 20 days or so, and then sprayed with a commercial mix of glufosinate at various concentrations ranging from about 0.1 to 1% active ingredient. The seedlings which contain the PAT gene (expression of which is determined by the method described by De Block M. et al (The EMBO Journal 6(9): 2513-2518 (1987)) are either completely resistant to glufosinate, or are relatively tolerant of the herbicide--depending upon the concentration applied--when compared with seedlings which do not contain the said gene.

Claims Text - CLTX (4):
4. A polynucleotide according to any preceding claim, wherein the proteins are selected from the group consisting of glyphosate oxido-reductase, 5-enol-pyruvyl-3-phosphoshikimate synthetase, phosphinothricin acetyl transferase, hydroxyphenyl pyruvate dioxygenase, glutathione S transferase, cytochrome P450, Acetyl-COA carboxylase, Acetolactate synthase, protoporphyrinogen oxidase, dihydropteroate synthase, polyamine transport proteins, superoxide dismutase, bromoxynil nitrilase, phytoene desaturase, the product of the tfdA gene obtainable from Alcaligenes eutrophus, and known mutagenised or otherwise modified variants of the said proteins.

PGPUB-DOCUMENT-NUMBER: 20020184661

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020184661 A1

TITLE: DNA SHUFFLING TO PRODUCE NUCLEIC ACIDS FOR MYCOTOXIN DETOXIFICATION

PUBLICATION-DATE: December 5, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
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APPL-NO: 09/ 414084

DATE FILED: October 6, 1999

CONTINUED PROSECUTION APPLICATION: This is a publication of a continued prosecution application (CPA) filed under 37 CFR 1.53(d).

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60103441 19981007 US

US-CL-CURRENT: 800/279, 435/419

ABSTRACT:

Methods of shuffling nucleic acids to acquire or enhance mycotoxin detoxification activity, libraries of shuffled mycotoxin detoxification nucleic acids, transgenic cells, plants and DNA shuffling mixtures are provided.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] The present application is a non-provisional of "DNA SHUFFLING TO PRODUCE NUCLEIC ACIDS FOR MYCOTOXIN DETOXIFICATION" by Subramanian, U.S. Ser. No. 60/103,441, filed Oct. 7, 1998.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (21):
[0019] The selected nucleic acids to be shuffled can be from any of a variety of sources, including synthetic or cloned DNAs. Exemplar targets for recombination include: nucleic acids encoding a monooxygenase, a P450, trichothecene-3-O-acetyltransferase, a 3-O-Methyltransferase, a glutathione S-transferase, an epoxide hydrolase, an isomerase, a macrolide-O-acytyltransferase, a 3-O-acytyltransferase, and a cis-diol producing monooxygenase which is specific for furan. Typically, shuffled nucleic acids are cloned into expression vectors to achieve desired expression levels.

Detail Description Paragraph - DETX (48):
[0095] For Glutathione S-trasferases (GSTs), the epoxide moiety of T2 is amenable to nucleophilic attack by thiol nucleophiles, including glutathione, whether transferred or not by GST. The thiol-T2 conjugate compound can be

formed in an irreversible manner and is not an active toxin. Endogenous GST levels in plants are likely to be sufficient. Selection in yeast is used to evolve and screen as above for 3-OAT. DNA shuffling is used to optimize the specificity of plant GST enzymes towards T2 epoxide.

Detail Description Paragraph - DETX (50):

[0097] Other assays for shuffling include chemical assays based on reactivity of residual epoxide, or formed rearranged 13-aldehyde product. One option is the use of a cytochrome P450 enzyme for aflatoxin detoxification by epoxidation of the double bond of the dihydrobisfuran moiety. Also, this can be used in conjunction with nucleophilic opening of the 15,16-epoxide (epoxide hydrolase, or GST, or an amine nucleophile, e.g. nucleobase or amino acid). Although 13-acetal can, in principle, be a subject to hydrolytic opening (enzymatic), the spontaneous toxophore regeneration may occur as it is favored by stereochemical means. Reactivity of 15,16-epoxide towards nucleophiles can be used for screen of P450s with the best activity towards AFB1. Exogenously supplied nucleophiles convenient for detection of AFB-epoxide-nucleophile adduct can be supplied in order to have shuffling done in bacterial or other microbial host which is insensitive to AFB1.

PGPUB-DOCUMENT-NUMBER: 20020177231

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020177231 A1

TITLE: LEUPAXIN MATERIALS AND METHODS

PUBLICATION-DATE: November 28, 2002

INVENTOR-INFORMATION:

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| LIPSKY, BRIAN P. | SEATTLE | WA | US | |
| GRAY, PATRICK W. | SEATTLE | WA | US | |


APPL-NO: 09/ 211424

DATE FILED: December 15, 1998

CONTINUED PROSECUTION APPLICATION: This is a publication of a continued prosecution application (CPA) filed under 37 CFR 1.53(d).

US-CL-CURRENT: 436/6, 530/350

ABSTRACT:

Disclosed are novel leupaxin polypeptides, polynucleotides encoding the polypeptides, expression constructs comprising the polynucleotides, host cell transformed with the polynucleotides, methods to produce the polypeptides, antibodies and binding partners specific for the polypeptides, methods to identify modulators of the polypeptides, and methods to identify modulators of polypeptide expression.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (18):

[0016] The invention also embraces leupaxin variants having additional amino acid residues which result from use of specific expression systems. For example, use of commercially available vectors that express a desired polypeptide such as a glutathione-S-transferase (GST) fusion product provide the desired polypeptide having an additional glycine residue at position -1 as a result of cleavage of the GST component from the desired polypeptide. Variants which result from expression in other vector systems are also contemplated.

Detail Description Paragraph - DETX (103):

[0099] Plasmids encoding each of the GST protein, the GST-LD fusion protein, the GST-LD fusion proteins with mutations in each LD motif, and the GST-92-106 protein were transformed into E. coli by standard methods and protein production was induced in exponentially growing cultures by addition of 0.5 mM IPTG for two hours. Cells were collected by centrifugation and lysed by sonication in D-PBS with 1 mM PMSF and 1% Triton-X-100. Clarified bacterial supernatant was incubated with glutathione agarose (Pharmacia) for 20 minutes at 4.degree. C., after which the resin was washed in D-PBS/1% Triton-X-100. Each of the various proteins was thereby immobilized on the resin.

Detail Description Paragraph - DETX (105):

[0101] Pyk2 present in lysates of JY8-8 cells bound specifically to GST-LD protein but not to GST alone. Pyk2 binding was detected with wild-type GST-LD, and GST-LD proteins with mutations in the second or fourth LD motifs. Pyk2 showed reduced binding to GST-LD with a mutation in the first LD motif and no binding to GST-LD with a mutation in the third LD motif The results indicated that the third LD motif was required for interaction with Pyk2. Consistent with this observation, Pyk2 binding was also detected with the fusion protein encoding leupaxin amino acid residues 92 to 106, encompassing the third LD motif, but not with GST alone.

PGPUB-DOCUMENT-NUMBER: 20020115225

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020115225 A1

TITLE: Microdevices for high-throughput screening of biomolecules

PUBLICATION-DATE: August 22, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
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APPL-NO: 10/ 112847

DATE FILED: March 29, 2002

RELATED-US-APPL-DATA:

child 10112847 A1 20020329

parent continuation-of 09115397 19980714 US PENDING

US-CL-CURRENT: 436/518

ABSTRACT:

Methods and devices for the parallel, in vitro screening of biomolecular activity using miniaturized microfabricated devices are provided. The biomolecules that can be immobilized on the surface of the devices of the present invention include proteins, polypeptides, nucleic acids, polysaccharides, phospolipids, and related unnatural polymers of biological relevance. These devices are useful in high-throughput drug screening and clinical diagnostics and are preferably used for the parallel screening of families of related proteins.

[0001] This application is a continuation of co-pending application Ser. No. 09/115,397, filed Jul. 14, 1998, which is incorporated herein by reference in its entirety for all purposes and the specific purposes disclosed throughout this application.

---------- KWIC ---------

Detail Description Paragraph - DETX (142):

[0172] Mutant proteases are generated by PCR mutagenesis (Weiner et al., Gene, 1994, 151:119) and expressed in Escherichia coli using two approaches: (i) mutant and wild-type protease cDNAs are cloned into a Escherichia coli expression vector containing a N-terminal histidine tag (H.sub.6; Hochuli et al., Biotechnology 1988, 6:1321) followed by a factor Xa cleavage site, while the stop codon of HIV protease is replaced by a sequence encoding a lysine tag

(K.sub.6) followed by a stop codon. The resulting fusion protein is purified from inclusion bodies as described in Wondrak and Louis, Biochemistry, 1996, 35:12957, and the histidine tag removed by factor Xa as described in Wu et al., Biochemistry, 1998, 37:4518; or (ii) mutant and wild-type protease cDNAs are cloned into an Escherichia coli expression vector creating a fusion between HIV protease, a tri-glycine linker, glutathione S-transferase (GST) and a lysine-tag (HIV-GST-K.sub.6). The autoprocessing site F*P at the carboxy terminus of the HIV protease is changed to F*I to prevent self-cleavage of the fusion proteins (Louis et al., Eur. J. Biochem., 1991, 199:361). The resulting proteins HIV-GST-K.sub.6 are purified from Escherichia coli lysates by standard chromatography on glutathione agarose beads and stored in an amine-free buffer at -80.degree. C. (25 mM HEPES, pH 7.5, 150 mM NaCl).

PGPUB-DOCUMENT-NUMBER: 20020110932

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020110932 A1

TITLE: Microdevices for screening biomolecules

PUBLICATION-DATE: August 15, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
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APPL-NO: 10/ 112982

DATE FILED: March 29, 2002

RELATED-US-APPL-DATA:

child 10112982 A1 20020329

parent continuation-of 09353554 19990714 US PENDING

child 09353554 19990714 US

parent continuation-in-part-of 09115397 19980714 US PENDING

US-CL-CURRENT: 436/518, 435/287.2 , 435/6

ABSTRACT:

Methods and devices for the parallel, in vitro screening of biomolecular activity using miniaturized microfabricated devices are provided. The biomolecules immobilized on the surface of the devices of the present invention include proteins, polypeptides, polynucleotides, polysaccharides, phospolipids, and related unnatural polymers of biological relevance. These devices are useful drug development, functional proteomics and clinical diagnostics and are preferably used for the parallel screening of families of related proteins.

[0001] This application is a continuation of co-pending application Ser. No. 09/353,554, filed Jul. 14, 1999, which is a continuation-in-part of co-pending application Ser. No. 09/115,397, filed Jul. 14, 1998, both of which are incorporated herein by reference in their entirety for all purposes and the specific purposes disclosed throughout this application.

---------- KWIC --------

Detail Description Paragraph - DETX (185):
[0213] Mutant proteases are generated by PCR mutagenesis (Weiner et al., Gene, 1994, 151:119) and expressed in Escherichia coli using two approaches:

(i) mutant and wild-type protease cDNAs are cloned into a Escherichia coli expression vector containing a N-terminal histidine tag (H.sub.6; Hochuli et al., Biotechnology 1988, 6:1321) followed by a factor Xa cleavage site, while the stop codon of HIV protease is replaced by a sequence encoding a lysine tag (K.sub.6) followed by a stop codon. The resulting fusion protein is purified from inclusion bodies as described in Wondrak and Louis, Biochemistry, 1996, 35:12957, and the histidine tag removed by factor Xa as described in Wu et al., Biochemistry, 1998, 37:4518; or (ii) mutant and wild-type protease cDNAs are cloned into an Escherichia coli expression vector creating a fusion between HIV protease, a tri-glycine linker, glutathione S-transferase (GST) and a lysine-tag (HIV-GST-K.sub.6). The autoprocessing site F*P at the carboxy terminus of the HIV protease is changed to F*I to prevent self-cleavage of the fusion proteins (Louis et al., Eur. J. Biochem., 1991, 199:361). The resulting proteins HIV-GST-K.sub.6 are purified from Escherichia coli lysates by standard chromatography on glutathione agarose beads and stored in an amine-free buffer at -80.degree. C. (25 mM HEPES, pH 7.5, 150 mM NaCl).

US-PAT-NO: 6683230

DOCUMENT-IDENTIFIER: US 6683230 B1

TITLE: Hybrid seed production

DATE-ISSUED: January 27, 2004

INVENTOR-INFORMATION:

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| Bayliss; Michael William | Haslemere | N/A | N/A | GB |

APPL-NO: 09/ 601812

DATE FILED: September 18, 2000

FOREIGN-APPL-PRIORITY-DATA:

| COUNTRY | APPL-NO | APPL-DATE |
|---|---|---|
| GB | 9803659 | February 20, 1998 |
| GB | 9805669 | March 17, 1998 |

PCT-DATA:
APPL-NO: PCT/GB99/00238
DATE-FILED: January 22, 1999
PUB-NO: WO99/42598
PUB-DATE: Aug 26, 1999
371-DATE:
102(E)-DATE:

US-CL-CURRENT: 800/271, 435/468 , 800/274 , 800/278 , 800/287 , 800/303 , 800/306 , 800/312 , 800/314 , 800/317.4 , 800/320 , 800/320.1 , 800/320.2 , 800/320.3 , 800/322

ABSTRACT:

Methods of preparing hybrid seed are described. One such method comprises interplanting a male parent plant which is male fertile and homozygous recessive female sterile and a female parent plant which is homozygous recessive male sterile and female fertile, allowing cross-pollination and obtaining the seed produced therefrom. The genomic material of each parent plant may also have integrated therein a gene construct comprising a promoter sequence-responsive to the presence or absence of an exogenous chemical inducer, optionally operably linked to one or more enhancer or intron sequences, operably linked to a gene which fully restores the fertility of each parent plant, the gene being expressed by the application to the plant of an external chemical inducer thereby allowing each parent to self-pollinate.

8 Claims, 68 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 42

---------- KWIC --------

Detailed Description Text - DETX (43):

The characterisation of the maize GST27 cDNA has been previously reported and experiments have shown that GST 27 is not constitutively expressed in silks, leaf, embryo or endosperm. After safener application, expression was detected in all of these tissues (see FIGS. 11 to 15). Plant transformation constructs utilising the GST 27 promoter to drive the expression of GUS may be made as described in U.S. Pat. No. 5,589,614 (FIG. 16). These are pGSTTAK (FIG. 17) for tobacco transformation and RMS-3 (FIG. 18) for corn transformation. These vectors could be used to generate stable tobacco and maize transforrnants. Formulated safener may be applied as either a leaf paint (tobacco) or as a root drench (corn) as described in the aforementioned patent and expression of GUS observed. The results for induction of GUS expression in tobacco leaves are shown in FIG. 12; clear induction of expression has occurred up to 100.times.. Similarly, for corn there has been an induction of GUS expression in leaf after safener treatment. Induction of expression was also observed in tassels and endosperm tissue and embryo.

Detailed Description Text - DETX (163):

REFERENCES Aarts et al., Transposon Tagging of a Male Sterility Gene in Arabidopsis. Nature, 363:715-717 Ahn, S, Tanksley, S. D, (1 993). Comparative linkage maps of the rice and maize genomes. Proc Nat Acad Sci 90: 7980-7984. Albani D., Robert L. S., Donaldson P. A., Altosaar I., Amison P. G., Fabijanski S. F. (1990) Characterization of a pollen-specific gene family from Brassica napus which is activated during early microspore development. Plant Mol. Biol. 15:605-622 Aoyama et al. (1995) Plant Cell 7, 1773 Atkinson, A. H., Heath, R. L., Simpson, R. J., Clarke, A. E., and Anderson, M. A. (1993). Proteinase inhibitors in Nicotiana alata stigmas are derived from a precursor protein which is processed into five homologous inhibitors. Plant Cell 5, 203-213. Becker D et al. Fertile transgenic wheat from microprojectile bombardment of scutellar tissue. Plant Journal 5: 299-307 (1994) Budelier K. A., Smith, A. G., and Gasser, C. S. (1990). Regulation of a stylar transmitting tissue-specific gene in wild type and transgenic tomato and tobacco. Mol. Gen. Genet 224, 183-192. Mark X. Caddick, Andrew J. Greenland, Ian Jepson, Klaus-Peter Krause, Nan Qu, Kay V. Riddell, Michael G. Salter, Wolfgang Schuch, Uwe Sonnewald and A. Brian Tomsett (1998) An ethanol inducible gene switch for plants used to manipulate carbon metabolism. Nature Biotechnology Vol16, 177-180. Paul Christou in Plant Mol Biol 35: 197-203 (1997) De Block, M., Debrouwer, T., Moens, T. (1997). The development of a nuclear male sterility system in wheat. Expression of the barnase gene under the control of tapetum specific promoters. Theor Appl Genet. 95, 125-131. H. P Doring (1989). Tagging genes with Maize Transposable elements. An Overview. Maydica 34 (1989) 73-88. Elliot, R. C., Betzner, A. S., Huttner E., Oakes, M. P., Tucker, W. Q. J., Gerentes, D., Perez, P., and Smyth, D. R. (1996) AINTEGUMENTA, an APELATA2-like gene of Arabidopsis with pleiotrophic roles in ovule development and floral organ growth. Plant Cell 8, 155-168 Ficker, M., Wemmer, T., and Thompson, R. D (1997). A promoter directing high level expression in pistils of transgenic plants. Plant Mol Biol 35, 425-431 Gallie, D. R, Sleat, D. E, Watts, J. W, Tumer, P. C, Wilson, T. M. A, (1987) A comparison of eukaryotic viral 5' leader sequences as enhancers of MnRNA expression in vivo. Nucleic Acids Research 15: 8693-8710. Gatz C et al. (1991) Mol Gen Genet 227, 229-237 Gatz C et al. (1992) Plant J 2, 397-404 Goldman, M. H., Goldberg, R. B., Mariane, C. (1994). Female sterile tobacco plants are produced by stigma specific cell ablation. EMBO J 13: 2976-2984. Hamilton, D. A., Bashe, D. M., Stinson, J. R., Mascarenhas, J. P. (1989). Characterisation of a pollen specific genomic clone from maize. Sex Plant Reprod 2, 208-212. Hanson, D. H., Hamilton, D. A., Travis, J. L., Bashe, D. M., Mascarenhas, J. P. (12989). Characterisation of a pollen specific cDNA clone from Zea Mays and its

expression. The Plant Cell 1, 173-179 Hasselhof. J and Gerlach W. L, 1988) Nature Vol 334: 585-591. Hiei Y et al. Efficient transformation of rice mediated by Agrobacterium. Plant Journal 6: 271-282 (1994) Hiei Y et al Rice transformation mediated by Agrobacterium tumefaciens. Plant Mol Boil 35: 205-218 (1997) Hird, D. L., Worrall, D., Hodge, R., Smartt, S., Paul, W and Scott, R (1993). The anther-specific protein encoded by the Brassica napus and Arabidopsis thaliana A6 gene displays similarity to .beta.-1,30glucanases. The Plant Journal 4(6), 1023-1033. Izawa T, Ohnishi T, Toshitsugu N, Ishida N, Hiroyuki E, Hashimoto H, Itoh K, Terada R, Wu C, Miyazaki C, Endo T, Iida S and Shimaamoto K (1997). Transposon tagging in rice. Plant Mol Biol 35: 219-229 Jepson, I, Lay, V., Holt, D. C., Bright, W. J., and Greenland, A. J., (1994). Cloning and characterisation of maize herbicide safener-induced cDNAs encoding subunits of glutathione S-transferase isoforms I, II and IV. Plant Molecular Biology 26: 1855-1866. Kamalay, J. C., and Goldberg, R. B. (1980). Regulation of structural gene expression in tobacco. Cell 19, 935-946 Kang H. G., Noh Y. S., Chung Y. Y., Costa M. A., An K., An G. (1995). Phenotypic alterations of petal and sepal by ectopic expression of a rice MADS box gene in tobacco. Plant Mol. Biol. 29:1-10 De Block, M., Debrouwer, T., Moens, T. (1997). The development of a nuclear male sterility system in wheat. Expression of the bamase gene under the control of tapetum specific promoters. Theor Appl Genet. 95, 125-131. Kempin, S. A., Liljigren, S. J., Block, L. M., Rounsley, S. D., and Yanofsky, M.F. (1997). Targeted disruption in Arabidopsis. Nature Vol 389 Kilian A, Chen J, Han F, Steffenson B and Kleinhofs A (1997). Towards map based cloning of the barley stem rust resistance genesRpgl and rpg4 using rice as an intergenomic cloning vehicle. Plant Mol Biol 35: 187-195. Koes R. E., Spelt C. E., van Den Elzen P. J. M., Mol J. N. M. (1989). Cloning and molecular characterization of the chalcone synthase multigene family of Petunia hybrida. Gene 81:245-257 Kozak, M (1989), J. Cell. Biol 108: 229-241 Kurata, N, Moore G, Nagamura Y, Foote T, Yano M, Minobe Y, Gale M. (1994) Conservation of genome structure between rice and wheat. Bio/Technology 12:276-278 Lloyd et al. (1994) Science 266, 436-439 Lotan, T., Ori, N., and Fluhr, R. (1989). Pathogenesis-related proteins are developmentally regulated in tobacco flowers. Plant Cell 1, 881-887 Mascarenhas D, Mettler I. J, Pierce D. A, Lowe, H. W (1990) Intron mediated enhancement of heterologous gene expression in maize. Plant Mol Biol 15: 913-920. Mariani, C., De Beuckeleer, Marc., Truettner, J., Leemans, J., and Goldberg, R. B (1990). Induction of male sterility in plants by a chimaeric ribonuclease gene. Nature Vol 347, 737-741 Celestina Mariani., Veronique Gossele., Marc De Beuckeleer., Marc De Block., Robert B. Goldberg., Willy De Greef and Jan Leemans (1992). A chimaeric ribonuclease-inhibitor gene restores fertility to male sterile plants. Nature Vol 357, 384-387. Merlo, Donald J.; Folkerts, Otto. (Dowelanco, USA). PCT Int. Appl., 117 pp. CODEN: PIXXD2. WO 9713402 A1 970417. Synthetic genes for d-endotoxins optimized for expression in plants and their in generation of lepidopteran-resistant plants. Mitchell and Tijan (1989) Science 245, 371-378. Moore G, Devos K. M, Wang Z, Gale M. D. (1995). Cereal genome evolution. Current Biol 5: 737-739 Nadeau, J. A., Zhang, X. S., Li, J and O'Neill, S. D. (1996). Ovule development: Identification of stage and tissue specific cDNAs. The Plant Cell 8, 213-239 Nacken et al: Mol and Gen Genet. 229, 129-136, (1991) Nasrallah, J. B., and Nasrallah, M. E. (1993). Pollen-stigma signalling in the sporophytic self-incompatibility response. Plant Cell 5., 1325-1335. Nelson, O. E., and Clary, G. B. (1952) J Hered. 43, 205-210 Ori, N., Sessa, G., Lotan, T., Himunelhoch, S., and Fluhr, R. (1990). A major stylar matrix polypeptide (sp41) is a member of the pathogenesis-realated proteins superclass. EMBO J 9, 3429-3436 Picard et al. (1993) TICB 3, 278-280 Ptashne (1988) Nature 355, 683-689 Ptashne and Gann (1990) Nature 346, 329-331 Robinson-Beers, K K., Pruitt, R. E., and Gasser, C. S. (1992). Ovule development in wild type Arabidopsis and two female sterile mutants. Plant Cell 4, 1237-1249 M. G. Salter, J. A. Paine, K. V. Riddell, I. Jepson, A. J. Greenland, M. X. Caddick and A. B. Tomsett (1998). Characterisation of the ethanol-inducible alc gene

expression system for transgenic plants. Plant Journal 16 (1) 127-132 Seed, Brian; Haas, Jurgen. (The General Hospital Corp., USA). U.S., 36 pp. Cont.-in-part of U.S. Ser. No. 324,243. CODEN: USXXAM. U.S. Pat. No. 5,795,737 A 980818. Patent written in English. Application: U.S. patent application Ser. No. 95-532390 950922. Priority: U.S. patent application Ser. No. 94-324243 940919 CAN 129:171505. Modulating efficiency of expression of foreign genes in host cells by manipulation of codon usage. Seurinck J., Truettner J., Goldberg R. B. (1990). Nucleotide sequence of an anther-specific gene. Nucleic Acids Res. 18:3403-3403 Simon et al. (I1996) Nature 384, 59-62 Spena, A., Estruch, J. J., Prensen, E., Nacken, W., Van Onckelen, H., Sommner, H. (1992). Anther-specific expression of the rolB gene of Agrobacteriun rhizogenes increases IAA content in anthers and alters anther development in whole flower growth. Theor Appl Genet 84, 520-527 Theissen G., Strater T., Fischer A., Saedler H. (1995). Structural characterization, chromosomal localization and phylogenetic evaluation of two pairs of AGAMOUS-like MAS-box genes from maize. Gene 156:155-166 Tsuchiya T., Toriyama K., Ejiri S., Hinata K. (1994). Molecular characterization of rice genes specifically expressed in the anther tapetum. Plant Mol. Biol. 26:1737-1746 Tsuchiya, T., Toriyama, K., Yoshikawa, M., Ejiri, S., Hinata, K., (1995). Tapetum specific expression of the gene for aan endo-.beta.-1,3-glucanase causes male sterility in transgenic tobacco. Plant Cell Phys. 36, 487-494. Twell, D., Wing, R. A., Yamaguchi, J., McCormick, S. (1989). Isolation and expression of an anther-specific gene from tomato. Mol Gen Genet 217, 240-245. Twell, D., Yamaguchi, J., McCormick, S. (1990). Pollen specific gene expression in transgenic plants: Coordinate regulation of two different tomato gene promoters during microsporogenesis. Development 109, 705-713. Vasil. V, Clancy. M, Ferl, R. J, Vasil. I. K, Hannah. L. C, (1989) Increased Gene Expression by the First I ntron of Maize Shrunken-1 locus IN Grass Species. Plant Phys 91: 1575-1579. Vasil V et al. Rapid production of transgenic wheat plants by direct bombardment of cultured immature embryos. Biotechnology 11:1553-1558 (1993). Weeks J T et al. Rapid production of multiple independant lines of fertile transgenic wheat. Plant Phys 102:1077-1084 (1993) D, Steinecke P, Herget T, Petereit I, Philipp C, Schreier P. H, (1994) Expression of a reporter gene is reduced by a ribozyme in transgenic plants. Mol Gen Genet 245: (465-470) Wright, S. Y., Suner, M-M., Bell, P. J., Vaudin, M., and Greenland, A. J (1993) Isolation and characterisation of male flower cDNA'S from maize. The Plant Journal 3, 41-49. Xu, H., Davis, S. P., Kwan, B. Y. H., O'Bries, A. P., Singh, M., Knox, R. P. (1993) Haploid and diploid expression of a Brassica Campestris anther specific gene promoter in Arabidopsis and tobacco. Mol Gen Genet 239, 58-65. Yokoi, S., Tsuchiya, T., Toriyama, K., Hinata, K (1997). Tapetum specific expression of the Osg6B promoter-.beta.-glucuronidase gene in transgenic rice. Plant Cell Reports 16, 363-367. Zou, J. T., Zhan, X. Y., WU, H. M., Wang, H., Cheung, A. Y. (1994). Characterisation of a rice pollen-specific gene and its expression. Am J Bot 81, 552-561. WO94/13822 Methods for stable transformation of wheat, Ciba Geigy.

US-PAT-NO: 6682942

DOCUMENT-IDENTIFIER: US 6682942 B1
**See image for Certificate of Correction**

TITLE: Microdevices for screening biomolecules

DATE-ISSUED: January 27, 2004

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---|---|---|---|---|
| Wagner; Peter | Belmont | CA | N/A | N/A |
| Ault-Riche; Dana | Palo Alto | CA | N/A | N/A |
| Nock; Steffen | Redwood City | CA | N/A | N/A |
| Itin; Christian | Menlo Park | CA | N/A | N/A |


APPL-NO: 09/ 570589

DATE FILED: May 12, 2000

PARENT-CASE:

This application is a continuation-in-part application of co-pending application Ser. No. 09/115,397, filed Jul. 14, 1998, which is incorporated herein by reference in its entirety.

US-CL-CURRENT: 436/518, 436/514 , 436/524

ABSTRACT:

Methods for the parallel, in vitro screening of biomolecular activity using miniaturized microfabricated devices are provided. The biomolecules immobilized on the surface of the devices employed in the method of the present invention include proteins, polypeptides, polynucleotides, polysaccharides, phospholipids, and related unnatural polymers of biological relevance. These methods are useful in drug development, functional proteomics studies and clinical diagnostics and are preferably used for the parallel screening of families of related proteins.

11 Claims, 9 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 8

---------- KWIC ---------

Detailed Description Text - DETX (185):
Mutant proteases are generated by PCR mutagenesis (Weiner et al., Gene, 1994, 151:119) and expressed in Escherichia coli using two approaches: (i) mutant and wild-type protease cDNAs are cloned into a Escherichia coli expression vector containing a N-terminal histidine tag (H.sub.6 ; Hochuli et al., Biotechnology 1988, 6:1321) followed by a factor Xa cleavage site, while the stop codon of HIV protease is replaced by a sequence encoding a lysine tag (K.sub.6) followed by a stop codon. The resulting fusion protein is purified from inclusion bodies as described in Wondrak and Louis, Biochemistry, 1996,

35:12957, and the histidine tag removed by factor Xa as described in Wu et al., Biochemistry, 1998, 37:4518; or (ii) mutant and wild-type protease-cDNAs are cloned into an Escherichia coli expression vector creating a fusion between HIV protease, a tri-glycine linker, glutathione S-transferase (GST) and a lysine-tag (HIV-GST-K.sub.6). The autoprocessing site F*P at the carboxy terminus of the HIV protease is changed to F*I to prevent self-cleavage of the fusion proteins (Louis et al., Eur. J. Biochem., 1991, 199:361). The resulting proteins HIV-GST-K.sub.6 are purified from Escherichia coli lysates by standard chromatography on glutathione agarose beads and stored in an amine-free buffer at -80.degree. C. (25 mM HEPES, pH 7.5, 150 mM NaCl).

US-PAT-NO: 6613959

DOCUMENT-IDENTIFIER: US 6613959 B1

TITLE: Transgenic plants expressing a MAPKKK protein kinase domain

DATE-ISSUED: September 2, 2003

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---|---|---|---|---|
| Sheen; Jen | Boston | MA | N/A | N/A |
| Kovtun; Yelena V. | Winchester | MA | N/A | N/A |
| Chiu; Wan-Ling | Richmond | VA | N/A | N/A |


APPL-NO: 09/ 371338

DATE FILED: August 10, 1999

PARENT-CASE:

This application claims benefit of U.S. provisional application Ser. No. 60/095,938 filed on Aug. 10, 1998.

US-CL-CURRENT: 800/278, 435/320.1 , 435/418 , 435/419 , 435/468 , 435/69.1 , 536/23.1 , 536/23.2 , 536/23.6 , 536/24.1 , 800/279 , 800/287 , 800/289 , 800/295 , 800/298 , 800/306 , 800/317

ABSTRACT:

The invention features a method for increasing stress resistance or tolerance in a plant, the method including the steps of: (a) introducing into plant cells a transgene including DNA encoding a kinase domain of a mitogen-activated protein kinase kinase kinase (MAPKKK) operably linked to a promoter functional in plant cells to yield transformed plant cells; and (b) regenerating a transgenic plant from the transformed cells, wherein the kinase domain of said MAPKKK is expressed in the cells of the transgenic plant, thereby increasing the level of stress resistance or tolerance in the transgenic plant. The invention further features plants including a recombinant transgene capable of expressing a kinase domain of a mitogen-activated protein kinase kinase kinase (MAPKKK) or a kinase domain thereof, wherein the transgene is expressed in said plant under the control of a promoter that is functional in a plant cell.

9 Claims, 26 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 26

---------- KWIC ---------

Detailed Description Text - DETX (22):

To further elucidate the molecular basis of oxidative stress signaling in plants, we have also showed that an Arabidopsis protoplast transient expression system is useful to investigate multiple stress responses. Three Arabidopsis

stress responsive promoters, glutathione S-transferase GST6 (Chen et al., Plant J. 10: 995-966, 1996), heat shock HSP18.2 (Takahashi and Komeda, Mol. Gen. Genet. 219: 365-372, 1989), and the abscisic acid (ABA) responsive promoter RD29A (Yamaguchi-Shinozaki et al., Plant Physiol. 101: 1119-1120, 1993; Ishitani et al., Plant Cell 9: 1935-1949, 1997), were fused to the luciferase (LUC) reporter and tested for their responses in transfected mesophyll protoplasts. The GST6, HSP18.2, and RD29A promoters were activated by H.sub.2 O.sub.2, heat, and ABA, respectively, in protoplasts (FIG. 5A) as demonstrated previously in intact plants (Chen et al., supra; Takahashi and Komeda, supra; Yamaguchi-Shinozaki et al., supra; Ishitani et al., supra). Several GST genes, including GST6, have been shown to be induced by high and toxic concentrations of plant growth hormone auxin, as well as by physiologically inactive auxin analogs, heavy metals, and numerous stresses (Chen et al., supra; Ulmasov et al., Plant Mol. Biol. 26: 1055-1064, 1994; Abel and Theologis, Plant Physiol. 111: 9-17, 1996; Sitbon and Perrot-Rechenmann, Physiol. Plantarum 100: 443-445, 1997; Guilfoyle et al., Plant Physiol., 118: 341-347, 1998, Marrs, Annu. Rev. Plant Physiol. Plant Mol. Biol. 47: 127-158, 1996). This non-specific induction of GSTs separates them from other auxin responsive genes that are only induced by low physiological levels of active auxin, and indicates that stress rather than auxin is responsible for the activation of the GST genes.

Detailed Description Text - DETX (35):

To determine whether a plant MAPKKK, such as ANP1 (Nishihama et al. Plant J. 12: 39-48, 1997), is involved in stress signal transduction, we have tested the effect of a constitutively active ANP1 kinase domain on the activity of several different dicot promoters. This was achieved by introducing into Arabidopsis protoplasts a transgene construct consisting of the firefly luciferase coding sequence (LUC) under the control of different dicot promoters. The promoters tested were the nitrate reductase, NR2, promoter from Arabidopsis (Lin et al., Plant Physiol. 106: 477-484, 1994); the asparagine synthetase, AS1, promoter (Neuhaus et al., EMBO J. 16: 2554-2564, 1997); the RD29A Arabidopsis stress-responsive promoter (Ishitani et al., Plant Cell 9: 1935-1949, 1997); the Arabidopsis HSP heat shock promoter (Sheen et al., Plant Journal 9: 777-784, 1995; Takahashi et al., Plant J. 2: 751-761, 1992); the Cab2 promoter (Mitra et al. Plant Mol. Biol. 12: 169-179, 1989); the chalcone synthase gene promoter (Feinbaum et al., Mol. Cell Biol. 8: 1985-1992, 1988); and the H.sub.2 O.sub.2 -inducible glutathione S-transferase promoter (GST) from Arabidopsis (Chen et al., Plant J. 10: 955-966, 1996). The kinase domain of ANP1 was cloned into a plant expression vector with a derivative of the 35S promoter and the nos terminator (Sheen, Science 274: 1900-1902, 1996). The ANP1 construct was co-transfected with one of the dicot promoter reporter gene construct and assayed according to standard methods. Surprisingly, the constitutively active ANP1 kinase domain was found to activate the expression of the AS1, HSP, and GST6 promoters (FIG. 7A). Constitutive expression of either the mutated NPK1 kinase domain or the CTR1 kinase domain had no effect on the expression of the dicot reporter genes.

Other Reference Publication - OREF (60):

Marrs, "The Functions and Regulation of Glutathione S-Transferases in Plants," Annu. Rev. Plant Physiol. Plant Mol. Biol. 47:127-158 (1996).

US-PAT-NO: 6582969

DOCUMENT-IDENTIFIER: US 6582969 B1

TITLE: Microdevices for high-throughput screening of biomolecules

DATE-ISSUED: June 24, 2003

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---|---|---|---|---|
| Wagner; Peter | Cupertino | CA | N/A | N/A |
| Ault-Riche; Dana | Palo Alto | CA | N/A | N/A |
| Nock; Steffen | Palo Alto | CA | N/A | N/A |
| Itin; Christian | Menlo Park | CA | N/A | N/A |

APPL-NO: 09/ 570586

DATE FILED: May 12, 2000

PARENT-CASE:

This application is a divisional application of co-pending U.S. patent application Ser. No. 09/115,397 filed on Jul. 14, 1998, which is incorporated herein by reference in its entirety.

US-CL-CURRENT: 436/518, 422/100 , 422/50 , 422/58 , 435/287.1 , 435/287.2 , 435/4 , 435/6 , 435/7.1 , 435/805 , 435/810 , 436/514 , 436/524 , 436/525 , 436/526 , 436/527 , 436/528 , 436/531 , 436/532

ABSTRACT:

Methods for the parallel, in vitro screening of biomolecular activity using miniaturized microfabricated devices are provided. The biomolecules that can be immobilized on the surface of the devices of the present invention include proteins, polypeptides, nucleic acids, polysaccharides, phospholipids, and related unnatural plyomers of biological relevance. These devices are useful in high-throughput drug screening and clinical diagnostics and are preferably used for the parallel screening of families of related proteins.

10 Claims, 10 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 10

---------- KWIC ---------

Detailed Description Text - DETX (142):

Mutant proteases are generated by PCR mutagenesis (Weiner et al., Gene, 1994, 151:119) and expressed in Escherichia coil using two approaches: (i) mutant and wild-type protease cDNAs are cloned into a Escherichia coli expression vector containing a N-terminal histidine tag (H.sub.6 ; Hochuli et al, Biotechnology 1988, 6:1321) followed by a factor Xa cleavage site, while the stop codon of HIV protease is replaced by a sequence encoding a lysine tag

(K.sub.6) followed by a stop codon. The resulting fusion protein is purified from inclusion bodies as described in Wondrak and Louis, Biochemistry, 1996, 35:12957, and the histidine tag removed by factor Xa as described in Wu et al., Biochemistry, 1998, 37:4518; or (ii) mutant and wild-type protease cDNAs are cloned into an Escherichia coli expression vector creating a fusion between HIV protease, a tri-glycine linker, glutathione S-transferase (GST) and a lysine-tag (HIV-GST-K.sub.6). The autoprocessing site F*P at the carboxy terminus of the HIV protease is changed to F*I to prevent self-cleavage of the fusion proteins (Louis et al., Eur. J. Biochem., 1991, 199:361). The resulting proteins HIV-GST-K.sub.6 are purified from Escherichia coli lysates by standard chromatography on glutathione agarose beads and stored in an amine-free buffer at -80.degree. C. (25 mM HEPES, pH 7.5, 150 mM NaCl).

US-PAT-NO: 6576478

DOCUMENT-IDENTIFIER: US 6576478 B1
**See image for Certificate of Correction**

TITLE: Microdevices for high-throughput screening of biomolecules

DATE-ISSUED: June 10, 2003

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---|---|---|---|---|
| Wagner; Peter | Cupertino | CA | N/A | N/A |
| Ault-Riche; Dana | Palo Alto | CA | N/A | N/A |
| Nock; Steffen | Palo Alto | CA | N/A | N/A |
| Itin; Christian | Menlo Park | CA | N/A | N/A |


APPL-NO: 09/ 115397

DATE FILED: July 14, 1998

US-CL-CURRENT: 436/518, 427/261 , 427/287 , 427/387 , 427/407.2 , 435/287.1 , 435/288.2 , 435/288.5 , 435/4 , 435/6 , 435/7.1 , 436/514

ABSTRACT:

Devices for the parallel, in vitro screening of biomolecular activity using miniaturized microfabricated devices are provided. The biomolecules that can be immobilized on the surface of the devices of the present invention include proteins, polypeptides, nucleic acids, polysaccharides, phospolipids, and related unnatural polymers of biological relevance. These devices are useful in high-throughput drug screening and clinical diagnostics and are preferably used for the parallel screening of families of related proteins.

10 Claims, 10 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 10

---------- KWIC ---------

Detailed Description Text - DETX (141):

Mutant proteases are generated by PCR mutagenesis (Weiner et al., Gene, 1994, 151:119) and expressed in Escherichia coli using two approaches: (i) mutant and wild-type protease cDNAs are cloned into a Escherichia coli expression vector containing a N-terminal histidine tag (H.sub.6, SEQ ID NO:1; Hochuli et al., Biotechnology 1988, 6:1321) followed by a factor Xa cleavage site, while the stop codon of HIV protease is replaced by a sequence encoding a lysine tag (K.sub.6, SEQ ID NO:2) followed by a stop codon. The resulting fusion protein is purified from inclusion bodies as described in Wondrak and Louis, Biochemistry, 1996, 35:2957, and the histidine tag removed by factor Xa as described in Wu et al., Biochemistry, 1998, 37:4518; or (ii) mutant and wild-type protease cDNAs are cloned into an Escherichia coli expression vector creating a fusion between HIV protease, a tri-glycine linker, glutathione S-transferase (GST) and a lysine-tag (HIV-GST-K.sub.6). The autoprocessing

site F*P at the carboxy terminus of the HIV protease is changed to F*I to prevent self-cleavage of the fusion proteins (Louis et al., Eur. J. Biochem., 1991, 199:361). The resulting proteins HIV-GST-K.sub.6 are purified from Escherichia coli lysates by standard chromatography on glutathione agarose beads and stored in an amine-free buffer at -80.degree. C. (25 mM HEPES, pH 7.5, 150 mM NaCl).

US-PAT-NO: 6500639

DOCUMENT-IDENTIFIER: US 6500639 B2

TITLE: DNA shuffling to produce nucleic acids for mycotoxin detoxification

DATE-ISSUED: December 31, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---|---|---|---|---|
| Subramanian; Venkiteswaran | San Diego | CA | N/A | N/A |

APPL-NO: 09/ 414084

DATE FILED: October 6, 1999

PARENT-CASE:

CROSS REFERENCE TO RELATED APPLICATIONS

The present application is a non-provisional of "DNA SHUFFLING TO PRODUCE NUCLEIC ACIDS FOR MYCOTOXIN DETOXIFICATION" by Subramanian, U.S. Ser. No. 60/103,441, filed Oct. 7, 1998.

US-CL-CURRENT: 435/69.1, 435/455 , 435/468 , 435/471 , 435/DIG.47 , 435/DIG.5 , 800/279

ABSTRACT:

Methods of shuffling nucleic acids to acquire or enhance mycotoxin detoxification activity, libraries of shuffled mycotoxin detoxification nucleic acids, transgenic cells, plants and DNA shuffling mixtures are provided.

21 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Brief Summary Text - BSTX (23):

The selected nucleic acids to be **shuffled** can be from any of a variety of sources, including synthetic or cloned DNAs. Exemplar targets for recombination include: nucleic acids encoding a monooxygenase, a P450, trichothecene-3-O-acetyltransferase, a 3-O-Methyltransferase, a **glutathione S-transferase**, an epoxide hydrolase, an isomerase, a macrolide-O-acytyltransferase, a 3-O-acytyltransferase, and a cis-diol producing monooxygenase which is specific for furan. Typically, **shuffled** nucleic acids are cloned into expression vectors to achieve desired expression levels.

Detailed Description Text - DETX (49):

For Glutathione S-trasferases (GSTs), the epoxide moiety of T2 is amenable to nucleophilic attack by thiol nucleophiles, including glutathione, whether transferred or not by **GST**. The thiol-T2 conjugate compound can be formed in an irreversible manner and is not an active toxin. Endogenous **GST** levels in

plants are likely to be sufficient. Selection in yeast is used to evolve and screen as above for 3-OAT. DNA shuffling is used to optimize the specificity of plant GST enzymes towards T2 epoxide.

Detailed Description Text - DETX (51):

Other assays for shuffling include chemical assays based on reactivity of residual epoxide, or formed rearranged 13-aldehyde product. One option is the use of a cytochrome P450 enzyme for aflatoxin detoxification by epoxidation of the double bond of the dihydrobisfuran moiety. Also, this can be used in conjunction with nucleophilic opening of the 15,16-epoxide (epoxide hydrolase, or GST, or an amine nucleophile, e.g. nucleobase or amino acid). Although 13-acetal can, in principle, be a subject to hydrolytic opening (enzymatic), the spontaneous toxophore regeneration may occur as it is favored by stereochemical means. Reactivity of 15,16-epoxide towards nucleophiles can be used for screen of P450s with the best activity towards AFB1. Exogenously supplied nucleophiles convenient for detection of AFB-epoxide-nucleophile adduct can be supplied in order to have shuffling done in bacterial or other microbial host which is insensitive to AFB1.

US-PAT-NO: 6077994

DOCUMENT-IDENTIFIER: US 6077994 A

TITLE: Genetic control of flowering

DATE-ISSUED: June 20, 2000

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
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APPL-NO: 08/ 945056

DATE FILED: October 20, 1997

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| COUNTRY | APPL-NO | APPL-DATE |
|---|---|---|
| GB | 9422083 | November 2, 1994 |

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DATE-FILED: November 1, 1995
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371-DATE: Oct 20, 1997
102(E)-DATE:Oct 20, 1997

US-CL-CURRENT: 800/290, 435/320.1 , 435/419 , 435/440 , 435/468 , 435/69.1 , 536/23.6 , 800/276 , 800/298 , 800/306

ABSTRACT:

The CONSTANS (CO) gene of Arabidopsis thaliana and homologues from Brassica napus are provided and are useful for influencing flowering characteristics in transgenic plants, especially the timing of flowering.

24 Claims, 6 Drawing figures

Exemplary Claim Number: 1,23

Number of Drawing Sheets: 9

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Brief Summary Text - BSTX (46):

As stated above, the expression pattern of the CO gene may be altered by fusing it to a foreign promoter. For example, International patent application WO93/01294 of Imperial Chemical Industries Limited describes a chemically inducible gene promoter sequence isolated from a 27 kD subunit of the maize glutathione-S-transferase, isoform II gene (GST-II-27) (See FIG. 2 (SEQ ID NO:3)). It has been found that when linked to an exogenous gene and introduced into a plant by transformation, the GST-II-27 promoter provides a means for the external regulation of the expression of that exogenous gene. The structural region of the CO gene is fused to the GST-II-27 promoter downstream of the

translation start point shown in FIG. 2 (SEQ ID NO:3).

Brief Summary Text - BSTX (48):

Accordingly, the present invention provides in a further aspect a gene construct comprising an inducible promoter operatively linked to a nucleotide sequence provided by the present invention, such as the CO gene of Arabidopsis thaliana, a homologue from another plant species or any mutant, derivative or allele thereof. This enables control of expression of the gene. The invention also provides plants transformed with said gene construct and methods comprising introduction of such a construct into a plant cell and/or induction of expression of a construct within a plant cell, by application of a suitable stimulus, an effective exogenous inducer. The promoter may be the GST-II-27 gene promoter or any other inducible plant promoter.

US-PAT-NO: 5965387

DOCUMENT-IDENTIFIER: US 5965387 A

TITLE: Promoter

DATE-ISSUED: October 12, 1999

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
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APPL-NO: 08/ 718751

DATE FILED: September 23, 1996

FOREIGN-APPL-PRIORITY-DATA:

| COUNTRY | APPL-NO | APPL-DATE |
|---|---|---|
| GB | 9519404 | September 22, 1995 |
| GB | 9519406 | September 22, 1995 |

US-CL-CURRENT: 435/69.1, 435/320.1 , 536/24.1

ABSTRACT:

A chemically inducible gene promoter sequence, and particularly, but not exclusively, a chemically inducible gene promoter sequence based on cis regulatory elements from the maize glutathione S-transferase 27 (GST-27) gene. In a preferred embodiment, the promoter sequence is operatively linked or fused to a gene or series of genes whereby expression of the gene or series of genes may be controlled by application of an effective exogenous inducer.

25 Claims, 19 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 19

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Drawing Description Text - DRTX (14):

FIG. 13 shows the results of a transient transformation assay of pPUG5 mutations. 500 .mu.l of BMS cells (50% packed cell volume) were transferred with 10 .mu.g plasmid DNA. Assays using each plasmid were carried out in triplicate under 2 conditions. 1 Induced media containing 40 ppm safener R-25788. 2 Uninduced media pPUG5 contains 570 bp of the GST-27 promoter and is known to retain inducibility. The 3 mutated constructs are sumilar to pPUG5 but each contains a 10 bp mutation around G residues which were footprinted at 24 hours after induction.

Detailed Description Text - DETX (14):

Primers were designed so that the area between -217 and -378 could be analysed using this method. A maize plant was treated with safener to induce

expression of GST-27. In-vivo footprint analysis was performed before the treatment (0 hours) and at 6, 24 and 48 hours after treatment. Results are shown in FIG. 8. It can be seen that a protein binds to a G residue at position -290 at 24 hours after the plant has been treated with safener (no band visible) but not at 0, 6 or 48 hours (band visible). Positions -275, -283 and -284 also have fainter bands at 24 hours. This result is reproducible. In short we have identified 2 putative elements which appears to bind protein factors in a safener dependent manner.

Detailed Description Text - DETX (67):
PCR-mediated Mutagenesis of GST-27 Promoter

Detailed Description Text - DETX (86):
The mutated fragments were introduced into the GST-27 promoter by cutting the pGEM-T vectors with HindIf and PstI and cloned into pPUG6 which had been linearised with the same enzymes. In this way the wild type promoter in pPUG6 was removed and replaced with the 3XMUT or 4XMUT version, so creating the plasmid p3XMUT/PUG6 or p4XMUT/PUG6. This was cloned into pBin400 using the same strategy as described above for the wild type pPUG6 plasmid, to form 3XMUT/Bin6 or 4XMUT/Bin6.

US-PAT-NO: 5856161

DOCUMENT-IDENTIFIER: US 5856161 A
**See image for Certificate of Correction**

TITLE: Tumor necrosis factor receptor-I-associated protein kinase and methods for its use

DATE-ISSUED: January 5, 1999

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
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APPL-NO: 08/ 580988

DATE FILED: January 3, 1996

PARENT-CASE:

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation in part of U.S. Ser. No. 08/445,410, filed May 19, 1995, now abandoned, which is a continuation in part of U.S. Ser. No. 08/271,424, filed Jul. 7, 1994, now abandoned.

US-CL-CURRENT: 435/195, 530/350 , 530/351

ABSTRACT:

The present invention provides an isolated and purified protein that associates with the cytoplasmic domain of the p60 form of the tumor necrosis factor receptor, having a molecular weight of about 52-55 kDa on SDS-PAGE, is a phosphoprotein, and does not bind to the p80 form of the tumor necrosis factor receptor. Also provided is an isolated and purified protein kinase that binds to the cytoplasmic domain of the p60 form of the tumor necrosis factor receptor, said kinase phosphorylates the p60 form of the tumor necrosis factor receptor. Also provided are various methods of manipulating this tumor necrosis factor receptor-associated protein and kinase in order to reduce various biological effects of tumor necrosis factor.

8 Claims, 33 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 33

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Drawing Description Text - DRTX (3):
FIG. 1 shows a schematic diagram of GST fusion proteins containing the cytoplasmic domain of the p60 form of the tumor necrosis factor receptor. The full-length p60 TNF receptor is shown with the extracellular domain (ED), the transmembrane region (shaded), and the cytoplasmic domain (CD). Residue numbering is based on the mature form of the receptor. Plasmids (left) and the

fusion proteins (right) expressed from them are indicated. GST-p60CD.DELTA.1 was used entirely. gg designates the glycine linker in the fusion protein GST-g-p60CD.

Drawing Description Text - DRTX (8):

FIG. 6 shows a schematic diagram of the deletion mutants of the cytoplasmic domain of the p60 TNF receptor. In FIG. 6A, the entire cytoplasmic domain (residues 205 to 426) of the p60 TNF receptor is shown as p60CD. The letters S/T/P and D/E designate the serine-, threonine- and proline-rich and acidic-residue rich regions as described below. Each deletion mutant was expressed as a fusion protein linked to GST as described below. In FIG. 6B, approximately equal amounts of the purified GST fusion proteins were analyzed by 9% SDS-PAGE and stained with Coomassie Blue. Molecular mass standards are expressed in kDa.

Drawing Description Text - DRTX (13):

FIG. 11 shows a schematic diagram of the deletion mutants of the cytoplasmic domain of the p60 TNF receptor. The entire cytoplasmic domain (residues 205 to 426) of the p60 TNF receptor is shown as p60CD. Each deletion mutant was expressed as a fusion protein linked to GST as described below. The shaded region indicates the death domain.

Drawing Description Text - DRTX (17):

FIG. 15 shows the deletions of the cytoplasmic domain of the p60 TNF receptor. FIG. 15A shows a schematic of the cytoplasmic domain of the p60 TNF receptor (residues 205-426) with the deletion constructs used herein. All deletions were expressed as GST fusion proteins. The site-specific mutants of p60.DELTA.8 were generated as described below. All deletion mutants bind p60TRAK activity. The shaded region indicates the death domain. FIG. 1B shows a SDS-polyacrylamide gel stained with Coomassie Blue of the GST fusion is shown with the indicated molecular mass standards. All fusion proteins were expressed and purified as described below.

Drawing Description Text - DRTX (21):

FIG. 19 shows the pervanadate modulates the activity of p60TRAK. FIG. 19A shows that pervanadate inhibits the activity of p60TRAK. U937 cells were incubated with the indicated concentration of pervanadate for 30 minutes at 37.degree. C. Cells lysates were prepared as described below and used for affinity precipitations with .about.5 .mu.g of GST, GST-p60.DELTA.8, or GST-p60.DELTA.12 bound to glutathione agarose. The precipitations were washed extensively, and in vitro kinase assays were performed as described below. The samples were analyzed by 9% SDS-polyacrylamide gel electrophoresis, and the dried gel exposed to X-ray film for 8 hours at -70.degree. C. The arrows indicate the migration of the GST fusion proteins, and the molecular mass standards are indicated in kDa. FIG. 19B shows that pp37 contains only phosphotyrosine. U937 cells were incubated with 100 .mu.M pervanadate for 30 minutes at 37.degree. C. Cell lysates were affinity precipitated with GST-p60.DELTA.8, and an in vitro kinase assay performed as described below. The sample was subjected to SDS-polyacrylamide gel electrophoresis and transferred to a PVDF membrane. The radioactive band corresponding to pp37 was excised from the membrane and a phosphoamino acid analysis was performed as described below. FIG. 19C shows that the amino acid sequence of the p60 cytoplasmic domain differs between p60.DELTA.8 and p60.DELTA.2. The underlined sequence is a consensus phosphorylation site (GT.sup.14 Y.sup.15 GVV) for the human WEE1 tyrosine kinase found in cyclin-dependent kinases (Nigg, 1995). FIG. 19D shows that in pervanadate-treated cells, Y.sup.331 undergoes phosphorylation by a tyrosine kinase that associates with GST-p60.DELTA.8. U937 cells were incubated in the presence or absence of 100 .mu.M pervanadate for 30 minutes at 37.degree. C. Cell lysates were prepared and affinity

precipitations were performed with the indicated wild type or mutant GST fusion protein as described below. In vitro kinase assays were conducted, and the samples analyzed by 8.5% SDS-polyacrylamide gel electrophoresis, and the dried gel was exposed to X-ray film for 3 hr at -70.degree. C. The relative mobility of GST-p60.DELTA.8, its site-directed mutants, and pp37 is indicated by the arrows. FIG. 19E shows that p49.sup.WEE1 phosphorylates Y.sup.331 of the p60 cytoplasmic domain in vitro. In vitro kinase assays with a volume (2 .mu.l, lanes 1, 3, 5, 7; or 10 .mu.l, lanes 2, 4, 6, 8) of purified p49.sup.WEE1 and with the substrates GST (lanes 1 and 2), GST-p60.DELTA.8 (lanes 3 and 4), GST-p60.DELTA.8.sup.T329A (lanes 5 and 6), or GST-p60.DELTA.8.sup.Y331F (lanes 7 and 8) was performed as described below. The proteins were resolved by 8.5% SDS-polyacrylamide gel electrophoresis, and the dried gel was analyzed by a PhosphorImager. FIG. 19F shows that GST-cdc25 dephosphorylates GST-p60.DELTA.8 that was phosphorylated by p49.sup.WEE1. GST-p60.DELTA.8 was phosphorylated by p49.sup.WEE1 in duplicate as described in FIG. 19E. The kinase assays were stopped with a kinase stop buffer and washed two times with phosphatase buffer. Purified GST-cdc25 was added to one of the reaction mixtures (lane 2), and the reaction was allowed to proceed for 20 minutes at 37.degree. C. The proteins were resolved by 8.5% SDS-polyacrylamide gel electrophoresis, and the dried gel was analyzed by a PhosphorImager.

Detailed Description Text - DETX (15):

FIG. 6 illustrates the fusion proteins that were made for this study. The construction of the plasmid encoding GST-p60 deletion mutants was as follows: The 5'-primers: CTAAGAGAATTCAGCTTCAGTCCCACT (SEQ.ID.NO.3) (.DELTA.2, .DELTA.3); CTAAGAGAATTCCCCAACTTTGCGGCT (SEQ.ID.NO.4) (.DELTA.4); and CTAAGAGAATTCACTGATGACCCCGCG (SEQ.ID.NO.5) (.DELTA.5); and the 3'-primers: TCTTAGTTAAGCTTAATCAGTCACCGGGGGTATA (SEQ.ID.NO.6) (.DELTA.2); TCTTAGTTAAGCTTAATCAGTCTAGGCTCTGTGG (SEQ.ID.NO.7) (.DELTA.3); and TCTTAGTTAAGCTTAATCATCTGAGAAGACT (SEQ.ID.NO.7) (.DELTA.4, .DELTA.5) were used to amplify fragments by PCR with unique restriction sites from pCMVXVBpL4-p60 of 0.10 kb (.DELTA.2), 0.25 kb (.DELTA.3), 0.46 kb (.DELTA.4), and 0.31 kb (.DELTA.5). The PCR products were digested with EcoRI/HindIII and inserted into digested pGEX-2TH to yield the expression vectors. Expression and purification of the GST fusion proteins from BL21 cells were carried out as described below. The amount of fusion protein was estimated by SDS-PAGE and also by staining with Coomassie Blue.

Detailed Description Text - DETX (48):

The present invention shows that a serine/threonine protein kinase associates with the cytoplasmic domain of the p60 form of the TNF receptor. In order to determine the region necessary for binding the kinase and the potential sites within the cytoplasmic domain that undergo phosphorylation, several deletion mutants of the cytoplasmic domain (FIG. 6) were constructed. The intracellular region of p60 form of the TNF receptor contains 222 aminoacyl residues with a large proportion of serine, threonine, proline, and acidic residues. Essentially, the cytoplasmic domain can be divided into two parts: 1) an N-terminal half that is rich in serine, threonine, and proline, and 2) a C-terminal half that contains a cluster of acidic residues. Therefore, GST fusion proteins containing deletions of the cytoplasmic region were generated to illustrate the functional role of these unique regions.

Detailed Description Text - DETX (49):

The examples above show that .DELTA.1 (243-426) is sufficient for binding kinase activity from U-937 cell lysate and also serves as a substrate for the associated kinase. Deletion mutant .DELTA.2 (243-274) contains the serine-, threonine- and proline-rich region, whereas .DELTA.4 (276-426) lacks it. Deletion mutants .DELTA.3 (243-323) and .DELTA.5 (324-426) are the N-terminal and C-terminal halves of the cytoplasmic domain, respectively. The C-terminal

acidic rich region is common to deletion proteins .DELTA.1, .DELTA.4, and .DELTA.5. In addition, the deletion protein .DELTA.5 is essentially the death domain as previously described. The GST fusion constructs were used to express the fusion proteins in E. coli; they were purified by affinity chromatography to glutathione-agarose (FIG. 6B). In order to minimize degradation of the fusion proteins in vivo, both .DELTA.1 and .DELTA.4 were expressed at 15.degree. C. while all others were expressed at 37.degree. C.

Detailed Description Text - DETX (52):
To illustrate the region necessary for binding and also for phosphorylation by the associated kinase, GST and the deletion mutants were used as both binding proteins and substrates for kinase reactions. Essentially, precleared cell lysates were bound to the desired fusion protein, washed extensively, and separated into tubes that contained substrates (GST or one of the deletion proteins) for i n vitro kinase reactions. The deletion proteins .DELTA.1, .DELTA.4, and .DELTA.5 were the only proteins capable of binding kinase activity and serving as substrates for the associated kinase (FIG. 7). GST neither bound any kinase activity nor served as a substrate in kinase reactions (FIG. 7). The amount of associated-kinase activity appeared to be highest with .DELTA.1 followed by .DELTA.5 and .DELTA.4, in order.

Detailed Description Text - DETX (110):
All plasmids encoding GST-p60 cytoplasmic domain deletions have been described above. The plasmids encoding site-directed mutants of GST-p60.DELTA.8.sup.T329A, GST-p60.DELTA.8.sup.Y331F and GST-p60.DELTA.8.sup.T329A/Y331F were generated by PCR using pCMVXVBpL4-p60 as the template and the following primers: .DELTA.8.sup.T329A (5'-BamHI): CTAAGAGGATCCACTGATGACCCCGCGGCGCTGTAC (SEQ.ID.NO.9); .DELTA.8.sup.Y331F and .DELTA.8.sup.T329A/Y331F (5'-BamHI): CTAAGAGGATTCACTGATGACCCCGCGG/ACGCTGTTCGCCGTGG (SEQ.ID.NO.10); and for all .DELTA.8 mutants (3'-HindIII): TCTTAGAAGCTTTTAGCGGAGCACGCGTCCCAG (SEQ.ID.NO.11). The PCR products were digested with BamHI-HindIII and inserted into pGEX2TH. The creation of a KspI site (underlined) in the primers made positive selection for the site-directed mutants possible.

Detailed Description Text - DETX (138):
A serine/threonine protein kinase associates with the cytoplasmic domain of the p60 TNF receptor, designated p60TRAK for TNF receptor-associated kinase. In addition, by deletion analysis the binding region of p60TRAK was further defined to residues 344-397 of the death domain. A region termed KID (kinase inhibitory domain) was also identified comprising residues 397-426 that inhibits the association of p60TRAK. For the present invention, GST fusion proteins of the cytoplasmic region of the p60 receptor that comprise deletions .DELTA.8, .DELTA.12, and .DELTA.15 were used, all of which lack the kinase inhibitory domain but contain the death domain (FIG. 15A). These three deletion mutants, however, differ in their N-terminus and thus in their size, which allowed distinguishing them from other substrates used. A Coomassie Blue stained SDS-polyacrylamide gel of the GST fusion proteins used is shown in FIG. 15B.

Detailed Description Text - DETX (164):
To demonstrate that Y.sup.331 is a potential site for phosphorylation, Y.sup.331 of GST-p60.DELTA.8 was replaced with phenylalanine by site-specific mutagenesis. Two additional mutants were also made: in one, T.sup.329 was replaced with alanine and in the other, a double mutant was made that contained both substitutions. These three mutant fusion proteins and wild-type GST-p60.DELTA.8 were used in affinity precipitations of cell lysates from control and pervanadate treated cells followed by in vitro kinase assays. pp37 did not appear in pervanadate-treated cell extracts that had been affinity

precipitated with GST-p60.DELTA.8.sup.Y331F or GST-p60.DELTA.8.sup.T329A/Y331F (FIG. 19D, lanes 6 and 8) but it did appear in those precipitated with both GST-p60.DELTA.8 (FIG. 19D, lane 2) and GST-p60.DELTA.8.sup.T329A (FIG. 19D, lane 4). In addition, as observed earlier with pervanadate treatment (FIG. 19A, lanes 3-7), the extent of phosphorylation of pp58 and pp55 decreased with p60TRAK bound to either GST-p60.DELTA.8 or its mutants (FIG. 19D). The mutations did not affect binding of p60TRAK to the cytoplasmic domain since the extent of phosphorylation of the fusion proteins was similar in untreated cells (FIG. 19D, lanes 1, 3, 5, and 7). Thus, these results suggest that there is a specific tyrosine site on the cytoplasmic domain of the p60 TNF receptor that undergoes phosphorylation.

Detailed Description Text - DETX (165):

Since the site of tyrosine phosphorylation in the p60 cytoplasmic domain is a putative site for phosphorylation by WEE1, whether GST-p60.DELTA.8 could be phosphorylated by WEE1 in vitro was examined. Bacterial expressed and purified p49.sup.WEE1 phosphorylated both GST-p60.DELTA.8 and GST-p60.DELTA.8.sup.T329A, but not GST or the mutant GST-p60.DELTA.8.sup.Y331F (FIG. 19E). In addition, the dual-specificity protein phosphatase cdc25, which has been implicated in the dephosphorylation of both T.sup.14 and Y.sup.15 of p34.sup.cdc2 (Gautier et al., 1991; Lee et al., 1992; Millar et al., 1991; Parker and Piwnica-Worms, 1992b), dephosphorylated GST-p60.DELTA.8 that had been phosphorylated by p49.sup.WEE1 (FIG. 19F). It has been shown that cdc25 serves as one of the substrates for CDKs (Hoffmann et al., 1993; Hoffmann et al., 1994; Izumi and Maller, 1993). Similarly, GST-cdc25 is also phosphorylated in vitro by p60TRAK (unpublished data), thus again suggesting that p60TRAK is similar to CDKs.

US-PAT-NO: 5843713

DOCUMENT-IDENTIFIER: US 5843713 A

TITLE: Peptide sequence that forms mucin sugar chain and technique for modifying protein to be linked with mucin sugar chain

DATE-ISSUED: December 1, 1998

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
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APPL-NO: 08/ 666473

DATE FILED: September 19, 1996

FOREIGN-APPL-PRIORITY-DATA:

| COUNTRY | APPL-NO | APPL-DATE |
|---|---|---|
| JP | 6-269111 | November 1, 1994 |
| JP | 7-022101 | February 9, 1995 |

PCT-DATA:
APPL-NO: PCT/JP95/02238
DATE-FILED: November 1, 1995
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PUB-DATE: May 9, 1996
371-DATE: Sep 19, 1996
102(E)-DATE:Sep 19, 1996

US-CL-CURRENT: 435/69.1, 435/320.1 , 435/325 , 435/70.1 , 536/23.1 , 536/23.4

ABSTRACT:

An amino acid sequence that can specifically introduce a mucin type sugar chain into a protein or peptide chain and a technique of introducing a mucin type sugar chain into protein or peptide by utilizing such a sequence are disclosed. GalNAc moiety of UDP-GalNAc (where UDP represents uridine 5'-diphosphate and GalNAc represents N-acetylgalactosamine) is introduced into the amino acid X(O) in the presence of UDP-GalNAc: polypeptide .alpha.1, O-GalNAc transferase (O-GalNAc T):

X(-1)-X(0)-X(1)-X(2)-X(3) (I)

where X(-1) and X(2) represent independently any amino acid, X(O) represents T or S and X(1) and X(3) represent independently any amino acid except that at least one of X(1) and X(3) represents P.

13 Claims, 26 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 23

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Drawing Description Text - DRTX (17):
FIG. 16 illustrates a construction of expression plasmid pGEX-3XS which is used for the production of a GST mutant including a peptide sequence having GalNAc acceptor activity at the C-terminal side of protein GST.

Detailed Description Text - DETX (134):
A recombinated gene coding for a mutant protein in which a peptide sequence having a GalNAc acceptor activity was inserted in a downstream region of GST-3X was constructed. The construction of the gene is illustrated in FIG. 11. The procedures for gene manipulation were according to the methods described in Molecular Cloning [J. Sambrook et al., Cold Spring Harbor Laboratory Press (1989)], unless otherwise noted.

Detailed Description Text - DETX (138):
Subsequently, 50 .mu.l of a solution containing 10 mM Tris-HCl (pH 8.0), 5 mM MgCl.sub.2, 100 mM NaCl, 1 mM 2-mercaptoethanol and 1 nmol of each of the above synthesized DNAs were prepared. The solution was then warmed to 75.degree. C. for 10 minutes and thereafter left to room temperature for annealing to produce a double-strand DNA, which was the desired DNA. A 5 .mu.l portion of the solution thus obtained was taken and the double-strand DNA was cut with EcoR I and BamH I and inserted between the same restriction enzyme sites of pGEX-3X to construct plasmid pGEX-3X Muc C1 according to a conventional method. The plasmid contained a DNA encoding the mutant, GST-3X Muc C1, n which MAAATPAPM was inserted between the 228th proline and the 229th glycine of the GST-3X. The sequence of the inserted region was confirmed by 373 A DNA sequencer (Applied Biosystems) with 5'pGEX Sequencing Primer (Pharmacia Biotech) and PRISM, Dye Terminator Cycle Sequencing Kit (Applied Biosystems).

Detailed Description Text - DETX (139):
The mutant protein containing the peptide sequence of MAAATPAPM (SEQ ID NO:105) inserted in a C-terminal region of GST-3X was prepared by utilizing E. coli in the following manner. E. coli BL 21 (Pharmacia Biotech) was transformed with pGEX-3X Muc C1 by means of the CaCl.sub.2 method and then precultured in 5 ml of 2.times.YTG culture medium (16 g/1 Tryptone, 10 g/l Yeast extract, 5 g/l NaCl, pH 7.0) containing 100 .mu.g/ml of ampicillin, which were precultured with shaking overnight at 37.degree. C. Subsequently, it was moved to 500 ml of the similar culture medium and cultured with shaking for 2.5 hours at 37.degree. C. (O.D.=0.5-1.0). A portion of 100 mM of Isopropyl-.beta.-D-thiogalactopyranoside (IPTG) was added to the culture solution to achieve a final concentration of 0.5 mM. The solution was then centrifuged at 5,000 rpm (4,470.times.g) for 10 minutes at 4.degree. C. to collect cells, which were then washed with 50 ml of 20 mM Tris-HCl (pH 7.5) and 140 mM NaCl and subjected to another centrifugation under the same conditions for collection. The cells were resuspended in 50 ml of 20 mM Tris-HCl (pH 7.5) and 140 mM NaCl and lyzed with a ultrasonic processer. The product was centrifuged at 15,000 rpm (27,700.times.g) for 30 minutes at 4.degree. C. and the supernatant was filtered by a membrane with a pore size of 0.22 .mu.m and 10% Triton X-100 was added to achieve a final concentration of 0.1%. The obtained solution was used as a crude enzyme solution.

Detailed Description Text - DETX (142):
The GalNAc transfer to GST-3X and GST-3X Muc C1 was analyzed according to the method described for determining the GalNAc acceptor activity of peptide except that 5 nmol of the GST-3X mutant was used instead of 100 nmol peptide.

Detailed Description Text - DETX (143):
FIG. 12 shows the results. As seen from FIG. 12, no substantial GalNAc transfer to GST-3X was observed, whereas GalNAc transfer to GST-3X Muc C1 increased as time went by. This fact shows that a protein can be altered to the mutant that can bind a mucin type sugar chain by inserting a peptide sequence having a GalNAc acceptor activity into the protein.

Detailed Description Text - DETX (152):
Finally, a plasmid of pGEY-3X 2A Muc N1 containing GST-3X 2A Muc C1 gene having a peptide sequence of MAAATPAP (SEQ ID NO:104) at the N-terminal was prepared in a manner as described below. A gene coding for the peptide sequence MAAATPAP (SEQ ID NO:104) was prepared by synthesizing single-strand Synthesized DNA 1 and Synthesized DNA 2 same as those of Example 11 and annealing them in 50 .mu.l of a solution containing 50 mM Tris-HCl (pH 7.5), 10 mM MgCl.sub.2, 100 mM NaCl and 1 mM 2-mercaptoethanol. In 5 .mu.l of the solution thus obtained, the double-strand DNA was cut by Nco I and inserted into the Nco I site of pGEY-3X 2A to produce plasmid pGEY-3X 2A Muc N1. The plasmid has MAAATPAP (SEQ ID NO:106) upstream to the methionine at the N-terminal of GST-3X and contains a DNA coding for a mutant, GST-3X 2A Muc N1, in which the serine at the second position of GST-3X had been changed to alanine. The sequence of the inserted region was confirmed by 373A DNA Sequencer (Applied Biosystems) with 5'-GTTGACAATTAATCATCCGGCTCGT-3' ((SEQ ID NO:106) synthesized and purified with HPLC by Kurasiki-Bouseki) and PRISM, Dye Terminator Cycle Sequencing Kit (Applied Biosystems).

Detailed Description Text - DETX (153):
The mutant protein GST-3X 2A Muc N1 was prepared by utilizing E. coli and analyzed as in the case of GST-3X Muc C1 described in Example 11. The GST-3X 2A Muc N1 which was detected in an eluted fraction from Glutathione Sepharose 4B column showed a GST activity equivalent to that of GST-3X. Further, it was detected as a single band with a molecular weight of about 28 K by SDS-PAGE.

Detailed Description Text - DETX (154):
The transfer of GalNAc to GST-3X 2A Muc N1 and to GST-3X was analyzed in the same manner as the method for measuring the GalNAc acceptor activity except that 5 nmol GST mutant was used instead of 100 nmol peptide.

Detailed Description Text - DETX (160):
In addition, GST-3X Muc C2, GST-3X Muc C3 and GST-3X Muc C4 were prepared by using a model protein of GST-3X as in the case of Example 11 but introducing respective peptide sequences that show a GalNAc acceptor activity and are different from that of GST-3X Muc C1 into the C-terminal side. For these mutants, GalNAc transfer activities were examined as in the case of the preceding two examples.

Detailed Description Text - DETX (175):
The mutant proteins GST-3X Muc C2, GST-3X Muc C3 and GST-3X Muc C4 were prepared by utilizing E. coli and analyzed in a manner as described for GST-3X Muc C1 in Example 11. Each of GST-3X Muc C2, GST-3X Muc C3 and GST-3X Muc C4 detected in eluted fractions from Glutathione Sepharose 4B column showed a specific GST activity comparable to that of GST-3X. Further, they were detected as a single band with a molecular weight of about 27 K by SDS-PAGE.

Detailed Description Text - DETX (176):
The transfer of GalNAc to each of the mutant GSTs was analyzed in the following manner, where SDS-PAGE and fluorography was combined. A 50 .mu.l of a solution (50 mM Imidazole-HCl (pH 7.2), 10 mM MnCl.sub.2, 0.5% Triton X-100 and 150 .mu.M UDP-[.sup.3 H]GalNAc) containing 5 nmol of the GST-3X mutant and 50 mU of partially purified 0-GalNAc T derived from colostrum of cow was

prepared and kept at 28.degree. C. for 20 hours. Then, 50 .mu.l of 2.times.SDS/sample buffer (125 mM Tris-Hcl (pH 6.8), 4% SDS, 4% 2-mercaptoethanol, 20% glycerol and 0.004% Bromophenol Blue) was added thereto and left in a boiling water for 5 minutes. Thereafter, 30 .mu.l of the reaction solution was applied to 12.5% SDS-PAGE. The gel was then immersed in a fixative solution (2-propanol/water/acetic acid (25:65:10)) for 30 minutes and then in Amprify (Amersham) for 30 min. Then, the gel was vacuum dried at 80.degree. C. and in close contacted with an X-ray film at -80.degree. C. for 15 days for exposure.

Detailed Description Text - DETX (190):

The insertion regions from pBEGST-3X and pBEGST-3X Muc C1 were cut out with restriction enzymes Xho I and Xba I and collected. Thereafter, they were inserted between the same restriction sites of plasmid vector pSVL for mammalian cells (Pharmacia Biotech) according to a conventional method to give pSEGST-3X and pSEGST-3X Muc C1. FIGS. 19A and 19B show restriction maps of the plasmids. It may be expected that, when the plasmids are introduced into a mammalian cell, EGST-3X and EGST-3X Muc C1 as the GST mutants which starts from the second serine from the N-terminal of the native GST-3X will be secreted into the culture.

Detailed Description Text - DETX (192):

The secreted GST mutant in the culture supernatant was purified with 1/2-scaled method, which was described in Example 11, concerning the E. coli culture with 0.5 ml of Glutathione Sepharose 4B column. The eluted fraction was condensed and changed to 10 mM potassium phosphate buffer (pH 6.2) with a Centricon-10 (Grace Japan).

Detailed Description Text - DETX (194):

In the treatment with glycosidases, neuraminidase derived from Arthrobacter ureafaciens (Boehringer Mannheim) and O-glycanase (Genzyme) derived from Diplococcus pneumoniae were used. The treatment with neuraminidase was conducted by adding 40 mU of the enzyme to about 300 ng of the GST mutant in 40 .mu.l of 20 mM potassium phosphate buffer (pH 6.2), followed by incubating the solution at 37.degree. C. for 13 hours. In the treatment of neuraminidase and O-glycanase, the reaction solution of neuraminidase described above was heated to 37.degree. C. for 1 hour, and then a 2mU of O-glycanase was added thereto before it was left for the reaction for 12 hours. As controls, a sample kept to 37.degree. C. for 13 hours and an untreated sample were prepared. Each of the samples was reacted with SDS by adding a same amount of 2.times.SDS/sample buffer and a 15 .mu.l of the reaction product was applied to SDS-PAGE. After electrophoresis, the gel was stained with 2D-silver staining reagent II "Daiichi" (Daiichi Pharmaceutical) to detect the protein band.

Detailed Description Text - DETX (201):

Example 14 showed that a GST mutant obtained by inserting a peptide sequence of MAAATPAPM (SEQ ID NO:105) was secretory expressed in COS7 cells and the produced EGST-3X Muc C1 had a typical mucin type sugar chain. Thus, in this example, each of GST-3X Muc C2, GST-3X Muc C3 and GST-3X Muc C4, that was confirmed to function, like GST-3X Muc C1, as a substrate for in vitro GalNAc transfer in Example 13, was fused with a signal peptide and secretory expressed in COS7 cells to confirm if the expressed proteins EGST-3X Muc C2, EGST-3X Muc C3 and EGST-3X Muc C4 can bind mucin type sugar chains. In addition, EGST-3X Muc C5 having a sequence of GTPGNSS, where amino acid at Position +1 is proline in the C-terminal region of EGST-3X, was also prepared.

Detailed Description Text - DETX (203):

After cutting pBEGST-3X with restriction enzyme Xba I, it was partially digested by restriction enzyme EcoR I to produce an about 0.9 kb DNA fragment

containing a structural gene with a complete length of EGST-3X. The fragment was then inserted between EcoR I and Xba I sites of pEF18S [T. Kato et al., J. Biochem, Vol.118, pp.229-236 (1995) and S. Mizushima et al., Nucleic Acid Res. Vol. 18, 5322(1990)] according to a conventional method to produce pEEGST-3X. pEF18S was used in expectation of a high level of expression, because the level of the expression of the GST mutant in the plasmid vector pSVL was not so high in Example 14.

Detailed Description Text - DETX (209):

Each of the prepared plasmids of pEEGST-3X, pEEGST-3X Muc C1, pEEGST-3X Muc C2, pEEGST-3X Muc C3, pEEGST-3X Muc C4 and pEEGST-3X Muc C5 was introduced into COS7 cells and the GST mutant that was secreted from the cells into the culture was purified and condensed in a manner as described in Example 14. The obtained EEGST-3X, EEGST-3X Muc C1, EGST-3X Muc C2, EGST-3X Muc C3, EGST-3X Muc C4 and EGST-3X Muc C5 showed a specific activity level comparable to that of GST-3X produced by E. coli. A Part of each sample was analyzed by 13% SDS-PAGE and silver staining to detect protein bands.

| Ref # | Hits | Search Query | DBs | Default Operator | Plurals | Time Stamp |
|---|---|---|---|---|---|---|
| L1 | 18311 | gst or glutathione s transferase$1 | US-PGPUB; USPAT | ADJ | OFF | 2005/02/03 10:46 |
| L2 | 220 | l1 near5 (plant or soy or glycine) | US-PGPUB; USPAT | ADJ | OFF | 2005/02/03 10:57 |
| L3 | 206 | 2 and (shuffl$ or muta$10) | US-PGPUB; USPAT | ADJ | OFF | 2005/02/03 10:49 |
| L4 | 2700 | 1 same (shuffl$ or muta$10) | US-PGPUB; USPAT | ADJ | OFF | 2005/02/03 10:49 |
| L5 | 27 | 2 and 4 | US-PGPUB; USPAT | ADJ | OFF | 2005/02/03 10:49 |
| L6 | 92 | l1 near5 (soy or glycine) | US-PGPUB; USPAT | ADJ | OFF | 2005/02/03 10:58 |
| L7 | 5381 | 1 near5 (gene$1 or sequence$1) | US-PGPUB; USPAT | OR | OFF | 2005/02/03 10:59 |
| L8 | 19 | 6 and 7 | US-PGPUB; USPAT | OR | OFF | 2005/02/03 10:59 |

PGPUB-DOCUMENT-NUMBER: 20050003512

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20050003512 A1

TITLE: Anti-bacterial vaccine compositions

PUBLICATION-DATE: January 6, 2005

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
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| Kennedy, Michael J. | Portage | MI | US | |

APPL-NO: 10/ 854299

DATE FILED: May 26, 2004

RELATED-US-APPL-DATA:

child 10854299 A1 20040526

parent division-of 09809665 20010315 US GRANTED

parent-patent 6790950 US

child 09809665 20010315 US

parent continuation-in-part-of 09545199 20000406 US PENDING

non-provisional-of-provisional 60153453 19990910 US

non-provisional-of-provisional 60128689 19990409 US

US-CL-CURRENT: 435/252.3

ABSTRACT:

Gram negative bacterial virulence genes are identified, thereby allowing the identification of novel anti-bacterial agents that target these virulence genes and their products, and the provision of novel gram negative bacterial mutants useful in vaccines.

[0001] This application is a continuation-in-part of U.S. patent application Ser. No. 09/545,199, filed Apr. 6, 2000, which claims benefit of U.S. Provisional Patent Application Ser. No. 60/153,453, filed Sep. 10, 1999 and 60/128,689, filed Apr. 9, 1999.

---------- KWIC ---------

Detail Description Paragraph - DETX (21):

[0041] To simplify the protein purification process, a purification tag may be added either at the 5' or 3' end of the gene coding sequence. Commonly used

purification tags include a stretch of six histidine residues (U.S. Pat. Nos. 5,284,933 and 5,310,663), a streptavidin-affinity tag described by Schmidt and Skerra, Protein Engineering, 6:109-122 (1993), a FLAG peptide [Hopp et al., Biotechnology, 6:1205-1210(1988)], glutathione S-transferase [Smith and Johnson, Gene, 67:31-40 (1988)], and thioredoxin [LaVallie et al., Bio/Technology; 11:187-193 (1993)]. To remove these peptide or polypeptides, a proteolytic cleavage recognition site may be inserted at the fusion junction. Commonly used proteases are factor Xa, thrombin, and enterokinse.

Detail Description Paragraph - DETX (27):

[0047] The invention also embraces variant polypeptides having additional amino acid residues which result from use of specific expression systems. For example, use of commercially available vectors that express a desired polypeptide as a fusion protein with glutathione-S-transferase (GST) provide the desired polypeptide having an additional glycine residue at position-1 following cleavage of the GST component from the desired polypeptide. Variants which result from expression using other vector systems are also contemplated.

PGPUB-DOCUMENT-NUMBER: 20040258694

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040258694 A1

TITLE: Hepatitis C receptor protein CD81

PUBLICATION-DATE: December 23, 2004

INVENTOR-INFORMATION:

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APPL-NO: 10/ 859700

DATE FILED: June 3, 2004

RELATED-US-APPL-DATA:

child 10859700 A1 20040603

parent continuation-of 09509612 20000329 US ABANDONED

child 09509612 20000329 US

parent a-371-of-international PCT/IB98/01628 19981006 WO UNKNOWN

FOREIGN-APPL-PRIORITY-DATA:

| COUNTRY | APPL-NO | DOC-ID | APPL-DATE |
|---|---|---|---|
| GB | 9721182.5 | 1997GB-9721182.5 | October 6, 1997 |
| GB | 9813560.1 | 1998GB-9813560.1 | June 23, 1998 |

US-CL-CURRENT: 424/161.1, 435/5 , 530/388.3

ABSTRACT:

The present invention relates to the use of CD81 protein and polynucleic acid in the therapy and diagnosis of hepatitis C and pharmaceutical compositions, animal models and diagnostic kits for such purposes.

---------- KWIC ---------

Brief Description of Drawings Paragraph - DRTX (11):

[0078] FIG. 8 represents the nucleotide and deduced amino acid sequence of the EC2-His.sub.6 fragment cloned into pGEX-KG as well as the upstream plasmid sequence coding for the carboxyl terminus of GST, the thrombin cleavage site and a small glycine spacer.

Detail Description Paragraph - DETX (49):

[0119] FIG. 8 represents the nucleotide and deduced amino acid sequence of the EC2-(His).sub.6 fragment cloned in pGEX-KG as well as the upstream plasmid sequence coding for the carboxyl terminus of GST, the thrombin cleavage site, and a small glycine spacer. As shown, EC2 is fused in frame with GST through the thrombin site, which can be exploited to remove GST from the fusion

protein. The glycine-rich spacer, located between thrombin site and EC2, facilitates the cleavage of the fusion protein by thrombin (Guan, K. L. and Dixon, J. E. (1991) Anal. Biochem. 192, 262-267).

Detail Description Paragraph - DETX (51):

[0121] The PCR product was digested with XhoI and HindIII, ligated to pGEX-KG (Guan, K. L., and Dixon, J. E. (1991) Anal. Biochem. 192, 262-267) digested with the same restriction enzymes, and transformed into TOP10 E. coli cells. After selection of the transformants by restriction enzyme analysis and nucleotide sequencing of the plasmids, a plasmid having the expected size of the insert was found to have also the correct EC2-(His).sub.6 sequence in frame with the upstream thrombin and GST coding sequence. The plasmid prepared from the selected TOP10 clone was then transformed into BL21 cells. Glycerol batches of selected clones were stored to -80.degree. C.

PGPUB-DOCUMENT-NUMBER: 20040110268

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040110268 A1

TITLE: ANTI-BACTERIAL VACCINE COMPOSITIONS

PUBLICATION-DATE: June 10, 2004

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
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APPL-NO: 09/ 809665

DATE FILED: March 15, 2001

RELATED-US-APPL-DATA:

child 09809665 A1 20010315

parent continuation-in-part-of 09545199 20000406 US PENDING

non-provisional-of-provisional 60153453 19990910 US

non-provisional-of-provisional 60128689 19990409 US

US-CL-CURRENT: 435/252.3

ABSTRACT:

Gram negative bacterial virulence genes are identified, thereby allowing the identification of novel anti-bacterial agents that target these virulence genes and their products, and the provision of novel gram negative bacterial mutants useful in vaccines.

[0001] This application is a continuation-in-part of U.S. patent application Ser. No. 09/545,199, filed Apr. 6, 2000, which claims benefit of U.S. Provisional Patent Application Serial Nos. 60/153,453, filed Sep. 10, 1999 and 60/128,689, filed Apr. 9, 1999.

---------- KWIC --------

Summary of Invention Paragraph - BSTX (44):

[0041] To simplify the protein purification process, a purification tag may be added either at the 5' or 3' end of the gene coding sequence. Commonly used purification tags include a stretch of six histidine residues (U.S. Pat. Nos. 5,284,933 and 5,310,663), a streptavidin-affinity tag described by Schmidt and Skerra, Protein Engineering, 6:109-122 (1993), a FLAG peptide [Hopp et al., Biotechnology, 6:1205-1210 (1988)], glutathione S-transferase [Smith and Johnson, Gene, 67:31-40 (1988)], and thioredoxin [LaVallie et al., Bio/Technology, 11:187-193 (1993)]. To remove these peptide or polypeptides, a

proteolytic cleavage recognition site may be inserted at the fusion junction. Commonly used proteases are factor Xa, thrombin, and enterokinase.

Summary of Invention Paragraph - BSTX (50):

[0047] The invention also embraces variant polypeptides having additional amino acid residues which result from use of specific expression systems. For example, use of commercially available vectors that express a desired polypeptide as a fusion protein with **glutathione-S-transferase (GST) provide the desired polypeptide having an additional glycine** residue at position-1 following cleavage of the GST component from the desired polypeptide. Variants which result from expression using other vector systems are also contemplated.

PGPUB-DOCUMENT-NUMBER: 20040110259

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040110259 A1

TITLE: Drug metabolizing enzymes

PUBLICATION-DATE: June 10, 2004

INVENTOR-INFORMATION:

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APPL-NO: 10/ 343593

DATE FILED: January 29, 2003

PCT-DATA:
APPL-NO: PCT/US01/24382
DATE-FILED: Aug 3, 2001
PUB-NO:
PUB-DATE:
371-DATE:
102(E)-DATE:

US-CL-CURRENT: 435/183, 435/252.3 , 435/320.1 , 435/325 , 435/69.1 , 536/23.2 , 800/8

ABSTRACT:

The invention provides human drug metabolizing enzymes (DME) and polynucleotides which identify and encode DME. The invention also provides expression vectors, host cells, antibodies, agonists, and antagonists. The invention also provides methods for diagnosing, treating, or preventing disorders associated with aberrant expression of DME.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (239):
[0232] In an alternative example, SEQ ID NO:7 is 90% identical to a human glutathione S-transferase subunit (GenBank ID g242749) as determined by BLAST analysis, with a probability score of 1.3e-101. SEQ ID NO:7 also contains glutathione S-transferase signature sequences as determined by searching for statistically significant matches in the M-based PFAM database of conserved protein family domains and by BIMPS analyses.

Detail Description Paragraph - DETX (85):
[0457] Protein arginine methyltransferase activity of DME is measured at 37.degree. C. for various periods of time. S-adenosyl-L-[methyl-.sup.3H]m-ethionine ([.sup.3H]AdoMet; specific activity=75 Ci/mmol; NEN Life Science Products) is used as the methyl-donor substrate. Useful methyl-accepting substrates include glutathione S-transferase fibrillarin glycine-arginine domain fusion protein (GST-GAR), heterogeneous nuclear rnbonucleoprotein (hnRNP), or hypomethylated proteins present in lysates from adenosine dialdehyde-treated cells. Methylation reactions are stopped by adding SDS-PAGE sample buffer. The products of the reactions are resolved by SDS-PAGE and visualized by fluorography. The presence, of .sup.3H-labeled methyl-donor substrates is indicative of protein argimne methyltransferase activity of DME (Tang, J. et al. (2000) J. Biol. Chem. 275:7723-7730and Tang, J. et al. (2000) J. BioL Chem.275:19866-19876).

PGPUB-DOCUMENT-NUMBER: 20040029151

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040029151 A1

TITLE: Molecular genetic profiling of gleason grades 3 and 4/5 prostate cancer

PUBLICATION-DATE: February 12, 2004

INVENTOR-INFORMATION:

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APPL-NO: 10/ 411537

DATE FILED: April 9, 2003

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60371304 20020409 US

US-CL-CURRENT: 435/6

ABSTRACT:

Many genes are affected in prostate cancers which have not been previously identified. This includes genes that have been up-regulated or down-regulated. Monitoring the expression levels of these genes is useful to identify the existence of prostate cancer. Also, monitoring the expression levels of these genes is useful to predict the effectiveness of treatment, outcome, use of therapeutics, and screening drugs useful for the treatment of prostate cancer.

RELATED APPLICATIONS

[0001] This application is a non-provisional of Application No. 60/371,304 filed on Apr. 9, 2002. This application is also related to U.S. Provisional Application No. 60/312,745, which is incorporated herein by reference for all purposes.

---------- KWIC ---------

Claims Text - CLTX (1):

1. A method for diagnosing prostate cancer in a patient, comprising the steps of: comparing level of expression of at least one RNA transcript or its translation product in a test sample of prostate tissue to level of expression of the at least one transcript or translation product in a control sample of

prostate tissue, wherein the test sample of prostate tissue is suspected of being neoplastic and the control sample is nonmalignant prostate tissue, wherein the at least one RNA transcript or its translation product is selected from a first or a second group of RNA transcripts or translation products, wherein the first group of RNA transcripts consists of transcripts of genes selected from the group consisting of GST alpha glutathione S-transferase exon 2 (X65727), Glutathione S-transferase Ha subunit 2 (GST) (M16594), transglutaminase (HG4020-HT4290), P15-protease inhibitor 5 (maspin) (U04313), L Arg:Gly amidinotransferase (S68805), KIAA0089 (D42047), RTVP-1 protein (X91911), GSTP1 (glutathione S-transferase pi) (M24485), L-arginine:glycine amidinotransferase (X86401), DNA endothelin-A receptor (D11151), Id1 (HG3342-HT3519), bcl-2 (M14745), Protein Phosphatase Inhibitor Homolog (HG3570-HG3773), pS2 protein (X52003), hAOX (aldehyde oxidase) (L11005), glutaredoxin (X76648), CO-029 (M35252), NADP dependent leukotriene b4 12-hydroxydehydrogenase (D49387), Glucocorticoid receptor alpha (M10901), Glucocorticoid receptor beta (HG4582-HT4987), ZAKI-4 from skin fibroblast (D83407), syndecan (exon 2-5) (Z48199), S-adenosylmethionine decarboxylase (M21154), hevin-like protein (X86693), gas 1 (L13698), pcHDP7 liver dipeptidyl peptidase IV (X60708), adult male liver squalene epoxidase (D78129), cathepsin H (X16832), oestrogen receptor (X03635), Id-related helix-loop-helix protein Id4 (U28368), apM2 GS2374 (D45370), macrophage capping protein (M94345), nucleotide binding protein (L04510), DNA cystatin A (D88422), Decorin (HG3431-HT3616), RACH1 (U35735), TIG2 (tazarotene-induced 2) (U77594), gravin (U81607), H19 RNA (M32053), adipsin/complement factor D (M84526), chondoitin sulfate proteoglycan versican V0splice-variant precursor peptide (U16306), nel-related protein 2 (D83018), IGFBP6 (insulin-like growth factor I) (X57025), cellular retinol-binding protein (M11433), laminin B1 chain (M61916), DNA primase (subunit 58) (X74331), complement protein component C7 (J03507), neuronal membrane glycoprotein M6b (U45955), TGF-beta3 (transming growth factor-beta3) (X14885), keratonocyte growth factor (M60828), SPARC/osteonectin (J03040), K+ channel beta subunit (L39833), procollagen C-proteinase enhancer protein (PCOLCE) (L33799), GTPase homolog HeLA cell line 833 nt (S82240), alpha-2 macroglobulin (M11313), thrombospondin (X14787), CAPL protein (M80563), prepro-alpha2(I) collagen (Z74616), pigment epithelium-derived factor (U29953), aspartoacylase kidney 1435 nt (S67156), class I alcohol dehydrogenase (ADH1) alpha subunit (M12963), CRBP (retinol binding protein) (X07438), Ovarian cancer down-regulated myosin heavy chain homolog (Doc1) (U53445), Insulin-like Growth factor 2 (HG3543-HT3739), Prostaglandin D2 synthase (M98539), hIRH (intecrin-alpha) (U19495), G9i) protein alpha-subunit (X04828), tryptase-III 3'-end (M33403), lumican (U21128), TIMP-3:C-terminus region (D45917), 3'UTR of unknown protein (Y09836), novel protein with short consensus repeats of six cysteines (U61374), h-SmLIM (smooth muscle LIM protein) (U46006), LACI (lipoprotein-associated coagulation inhibitor) (M59499), phospholamban (M63603), transcriptional activator hSNF2a (D26155), smooth muscle myosin heavy chain (D10667), erm exon2,3,4,5 (X96381), telomeric repeat binding factor (TRF1), N2A3 (U97105), GBP-2 (guanylate binding protein isom I) (M55542), metalloproteinase inhibitor (M32304), matrilin-2 precursor, 11-HSD11 (beta-hydroxysteroid dehydrogenase) (M76665), CCK (cholecystokinin) (L00354), apM2 GS2374 (D42047), CYP1B1 (dioxin-inducible cytochrome P450) (U03688), lung amiloride sensitive Na+ channel protein (X76180), PCP4 (PEP19) (U52969), NAT1 (anylamine N-acetyltransferase) (X17059), squalene synthase (X69141), Id-2 (helix-loop-helix protein) (M97796), Zn-alpha2-glycoprotein (X59766), Striated muscle contraction regulatory protein (Id2B) (M96843), Glucocorticoid receptor Beta (HG4582-HT4087), HLH 12R1 helix-loop-helix protein (X69111), PSE-binding factor PTF gamma subunit (U44754), cancellous bone osteoblast GS3955 (D87119), prostatic secretory protein 57 (U22178), K-sa, (Fibroblast Growth Factor Receptor) (M87770), creatine kinase-B (M16364), ornithine aminotrasnferase (M29927), epsilon-BP (IgE-binding protein) (M57710), ARL3 (GTP binding protein) (U07151), RNase 4 (D37921), MSP (Beta-microsemiprotein) (M34376), phospholipase

C (D42108), lipocortin II (D00017), DBI (diazepam binding inhibitor) (M14200), KIAA0367 (AB002365), MAT8 protein (X93036), protein-tyrosin phosphatase (HU-PP-1) (U14603), imogen38 (Z68747), Cystatin A (D88422), Cytokeratine 15 (X07696), P-450 HFLa (Fetal liver cytochrome P450) (D00408), Fetal brain (239FB) mRNA from the WAGR region (U57911), Caveolin (Z18951), MLCK (myosin light chain kinase) (U48959), cardiac gap junction protein (X52947), lactate dehydrogenase B (Ec 1.1.1.27) (X13794), KIA0003 (D13628), TRPC1 protein (X89066), unknown protein (D28124), K+ channel beta subunit (L39833), COX7A (cytochrome c oxidase subunit VIIa muscle isoforms (M83186), desmin (M63391), HBNF-1 (nerve growth factor) (M57399), hIRH intercrien-alpha (U19495), fibroblast muscle-type tropomyosin (M12125), SLIM1 (skeletal muscle LIM-protein) (U60115), Adipsin/complement factor D (M84526), Epidermalkeratin-50 kDa type Ie (J00124), H-19 RNA (M32053), Keratin type II 58 kD (M21389), neuronal membrane glycoprotein M6B (U45955), GS TM3 (Glutathione transferase M3) (J05459), unknown protein (U61374), Insulin-like growth factor-2 (IG3543-HT373), IGFBP6 (insulin-like growth factor binding protein 6) (M62402), P-cadherin (X63629), alpha-B crystalline (S45630), MaxiK potassium channel beta (U25138), MLC-2 (myosin light chain) (J02854), caveolin 2 (U32114), SOD3 (extracellular superoxide dismutase) (J02947), ERM (X96381), GLUT5 (Glucose transport-like 5) (M55531), pigment epithelium derived factor (U29953), CRBP (retinol binding protein) (X07438), calcyclin (IG2788-HT289), dehydropyrimidinase related protein-3 (D78014), NECDIN related protein (U35139), CAPL protein (M80563), Mig-2 (Z24725), Heat shock protein 28 kDa (Z23090), smooth muscle gamma-actin (D00654), p68 (Y00097), KIAK002 (D13639), G9i) protein-alpha subunit (adelynate cyclase inhibiting GTP-b) (X04828), BPAG1 (Bullous pemphigoid antigen) (M69225), retinol-binding protein (M11433), TGF beta (transming growth factor-beta type III receptor) ((L07594), aspartoacylase (S67156), ERF-2 (X78992), complement protein component C7 (J03507), Mac-2 binding protein (L13210), vinculin (M33308), phospholamban (M63603), tissue inhibitor of metalloproteinase 3 (U14394), calponin ((D17408), glypican (hepara sulfate proteoglycan (X54232), keratinocyte growth factor (M60828), trophinin (U04811), TRPM-2 protein (M63379), filamin ABP-280 (actin binding protein) ((X53416), collagen VI alpha 2C-terminal globular domain (X15882), GBP-2 (guanylate binding protein II) (M55543), CALLA (common acute lymphoblastic leukemia antigen) (J03779), enigma ((L35240), MT-11 (X76717), ALDHI (RNA mitochondrial aldehyde dehydrogenase) (X05409), breat tumor antigen (U24576), non-muscle alpha-actinin (M95178), pur (pur-alpha) (M96684), N2A3 (U97105), 64 kD autoantigen expressed in thyroid and extra-occular muscle (X54162), GTPase homolog (S82240), arginase type II (U82256), tryptase-III (M33493), CD38 (D84276), muscarinic acetylcholine receptor (M35128), NF-H exon 1 (X15306), tenascin-C 7560 bp (X78565), LPP (IIM protein) (U49957), KIA0172 (D79994), MTIG (clone 14 VS metallothionein-IG) (J03910), smoothelin (Z49989), KIP 2 (Cdk-inhibitor p57 KIP1 (U22398), n-chimaerin (X51408), metallothionein from cadmium-treated cells (V00594), collagen VI alpha-IC-terminal globular domain (X15880), soluble carrier family 39 (zinc transporter) (NM.sub.--014579.1), secretoglobin family IA member I (uteroglobin) (NM.sub.--003357.1), serine or cystrein proteinase inhibitor (NM.sub.--002639.1), SIAT7E (NM.sub.--030965), nebulin (NM.sub.--004543.2), proenkephalin (NM.sub.--006211.1), aminolevulinate delta dehydratase (BC000977.1), hypothetical protein FIJ20513 (NM.sub.--017855.1), erythrocyte membrane protein band 4.1-like 3 (AI770004), adipose specific 2, unknown protein (BG109855), syndecan 1 (Z48199), keratin 5 (NM.sub.--000424.1), cytochrome p450 subfamily 1 (NM.sub.--000104.2), glutathione S-transferase pi (NM.sub.--000852.2), phosphorylase glycogen (NM.sub.--002863.1), zinc finger protein 185 (LIM domain) (NM.sub.--007150.1), single carrier family 16 AA705628), aminoethyltransferase (NM.sub.--000481), transmembrane 7 superfamily member 2 (AF096304.1), chemokine (C-X-C motif) ligand 13 (NM.sub.--006419.1), NEL-like 2 (NM.sub.--006159.1), D component of complement (adipsin) (NM.sub.--001928.1), EGF-containing fibulin-like EMP-1 (AI826799), retinol binding protein 1 (NM.sub.--002899.2), fibulin 1 (Z95331),

tissue inhibitor of metalloproteinase 3 (NM.sub.--000362.2), signal transduction protein (NM.sub.--005864.1), dihydrpyramidinase-like 3 (NM.sub.--001387.1), WNT inhibitory factor 1 (NM.sub.--007191.1), signal transduction protein (SH3 containing)(NM.sub.--005864.1), collagen type IV alpha 6 (AI889941), suppression of tumorigenicity 5 (NM.sub.--005418.1), and wherein the second group of RNA transcripts or translation products are being selected from the group consisting of pyrroline 5-carboxylase reductase (M77836), KIAA0230 (D86983), transcription factor ETR101 (M62831), TGF-beta superfamily (AB000584), intestinal trefoil factor (L08044), aldehyde dehydrogenase 6 (U07919), carcinoma associated antigen GA733-2 (M93036), IQGAP2 (RasGAP-related protein) (U51903), Macmarcks (HG1612-HT1612), KIAA0056 (D29954), SOX-4 protein (X70683), hR-PTPu protein tyrosine phosphatase (X58288), EGR2 (early growth response 2) (J04076), DNApolymerase gamma (U60325), cystathionine beta synthase, alt splice 3 (HG2383-HT4824), CPBP (DNA-binding protein CPBP) (U44975), skeletal muscle C-protein (X66276), HU-K5 (lysophospholipase homolog) (U67963), fibromodulin (U05291), prostatin (L41351), apolipoprotein E (M12529), hEGR1 (early growth response 1) (X52541), DNA polymerase beta (D29013), GOS3 (L49169), ANK-3 (Ankyrin G) (U13616), Gap junction protein (X04325), Hepsin (X07732), CYP1B1 (dioxin-inducible cytochrome P450 (U03688), T-cell receptor Ti rearranged gamma chain V-J-C (M30894), KIAA00167 (D28589), omithine decarboxylase (M33764), Tob (D38305), 17-beta-hydroxysteroid dehydrogenase (X87176), homeo box c8 protein (M16938), TRAIL (TNF-related apoptosis inducing ligand (U37518), cellular onco-fos (V01512), ESE-1 (epithelial-specific transcription factor) (U73843), prostate-specific membrane antigen, alternatively spliced (S76978), prostate-specific membrane antigen (M99487), T-cell receptor Ti rearranged gamma chain V-J-C region (M30894), OSF-2os (Osteoblast specific factor 2) (D13666), LDL phospholipase A2 (U24577), MAOA (monoamine oxidase A) (M68840), ALCAM (CD6 ligand) (L38608), UDP-GalNAC:polypeptide N-acetylgalactosaminyl transferase (X92689), NB thymosin beta (D82345), FBPI (Fructose-1,6 biphosphatase), NMB (X76534), cytochrome c-1 (J04444), ionizing radiation conferring protein (U18321), Myoglobin exon 1 (X00371), Memc (U30999), Clone 23587 sequence (U90914), pyrroline 5-carboxylate synthetase (X94453), ADE2H1 (X53793), (SNX) sorting nexin 1 (U53225), IMPDH2 (inosine monophospate dehydrogenase type II) (L33842), transcription factor E2F-5 (U31556), propionyl CoA carboxylase beta subunit (S67325), 6-pyruvoyl-tetrahydropterin synthase (D17400), ADP/ATP carrier protein (J02683), nucleoside diphosphate kinase Nm23-H2s (HG1153-HT1153), ornithine decarboxylase (M33764), CLCN3 (X78520), c-fos (V01512), PCC (propionyl-CoA carboxylase bea-subunit) (M31169), adenylsuccinate lyase (X65867), Cctg chaperonine (X74801), SIM2 (U80456), liver gap-junction protein (X04325), C-myc (L00058), HLA-DMB (U15085), carcinoma-associated antigen GA733-2 (M93036), homeo box c8 protein (M16938), GST-1 Hs GTP binding protein (X17219), Brain guanine nucleotide binding protein (M17219), spermidine synthase (M34338), NAD-dependent methylene tetrahydrofolate dehydrogenase cyclohydrolase (E.C. 1.5.1.15) (X16396), C8FW phosphoprotein (AJ000480), NBK apoptotic inducer protein (X89986), TK (t-ansketolase) (L12711), MNK1 (AB000409), fatty acid synthase (S80437), tubulin beta (HG4322-HT4592), testican (X73608), Arg protein kinasebinding protein (X95632), DNA polymerase delta (U21090), IP-30 (gamma-interferoninducible protein) (J03909, Lutheran blood group glycoprotein (X83425), tyrosine phosphatatase 1 non-receptor (HG3187-HT3366), mestatasis-associated mta-1 (U35113), (RPS6KA2) ribosomal protein S6 kinase 2 (L06797), transcription factor mef2 alt. splice 2 (HG4668-HT5083), basic transcription factor 44 kDa (HG3748-HT4018), soluble guanylate cyclase large subunit (X66534), transcription factor ETR101 (M62831), orphan G-protein-coupled receptor (L06797), MHC Class II W52 (HG3576-HT3779), prostasin (L41351), M6 antigen (X64364), Mrp17 (X79865), Ly-GDI (GDP-dissociation inhibitor protein) (L20688), KH type splicing regulatory protein KSRP (U94832), Ia-associated invariant gamma-chain (M13560), HLA-DRB1

(MHC class II beta1) (M33600), transcriptional activator hSNF2b (D26156), USF2 (AD000684), SEP protein (X87904), nested protein (M34677), HOXA9 (class I homeoprotein) (U41813), BRG1 (transcriptional activator) (U29175), KIAA0075 (D38550), eIF3 (translational initiation factor) (U78525), KIAA0113 (D30755), HU-K5 (lysophospholipase homolog) (U67963), ADP/ATP tranlocase (J03592), inducible poly(A)-binding protein (U33818), KIAA0146 (D63480), NET1 (guanine nucleotide regulatory protein) (U02081), KIAA0162 (D79984), v-ets erythroblastosis virus E26 oncogene like (AI351043), FBJ murine osteosarcroma viral oncogene homolog B (NM.sub.--006732.1), ubiquitin D (NM.sub.--006398.1), sialyltransferase I (AI743792), RALBP1 associated Eps domain containing 2 (NM.sub.--004726.1), chemokine (C-C motif) ligand 19 (U88321.1), transient receptor potential cation channel subfamily M member (NM.sub.--01736.1), B cell activation gen (S59049.1), eukaryotic translation initiation factor 4E binding protein 1 (AB044548.1), lymphocyte antigen 75 (NM.sub.--002349), alpha-methylacyl-CoA racemase (NM.sub.--014324.1), phosphoprotein regulated by mitogenic pathway (NM.sub.--025195.1), RALBP1 associateds Eps domain containing 2 (NM.sub.--004726.1), neuropilin (NRP) and tolloid (TLL)-like 2 (NM.sub.--018092.1), twist homolog (X99268.1), calcium calmodulin-dependent protein kinase 2 (AA1181179), tumor associated calcium signal transducer 1 (NM.sub.--002354.1), UDP-N-acetylglucosamine phosphorylase I (S73498.1), epithelial cell transforming sequence 2 oncogene (NM.sub.--01898.1), myosin VI (U90236.2), LIM protein (NM.sub.--006457.1), claudin 8 (AL049977.1), phosphoprotein regulated by mitogenic pathway (NM.sub.--025195.1), thymosin beta (NM.sub.--021992.1), TNF (ligand) superfamily (U57059.1), unknown protein (AV715767), activated leucocyte cell adhesion molecule (NM.sub.--001627.1), chaperonin containing TCP1 (NM.sub.--001762.1), phosphoribosylaminoimidaz- ole carboxylase (AA902652), protein (NM23A) (NM.sub.--000269.1); and identifying the test sample as cancerous when expression of at least one of the first group of RNA transcripts or translation products is found to be lower in the test sample than in the control sample, and expression of at least one of the second group of transcripts or translation products is found to be higher in the test sample than in the control sample.

PGPUB-DOCUMENT-NUMBER: 20030023055

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030023055 A1

TITLE: ATR-2 cell cycle checkpoint

PUBLICATION-DATE: January 30, 2003

INVENTOR-INFORMATION:

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APPL-NO: 09/ 957837

DATE FILED: September 21, 2001

RELATED-US-APPL-DATA:

child 09957837 A1 20010921

parent division-of 09417822 19991014 US PATENTED

US-CL-CURRENT: 536/23.1

ABSTRACT:

Polynucleotides encoding novel Atr-2 cell cycle checkpoint polypeptides are disclosed, along with expression constructs comprising the polynucleotides, host cells transformed with the expression constructs, methods to make the Atr-2 polypeptides using the host cells, Atr-2 polypeptides, and binding partners of the Atr-2 polypeptides.

---------- KWIC --------

Summary of Invention Paragraph - BSTX (72):

[0068] The invention also embraces Atr-2 variants having additional amino acid residues which result from use of specific expression systems. For example, use of commercially available vectors that express a desired polypeptide as part of glutathione-S-transferase (GST) fusion product provides the desired polypeptide having an additional glycine residue at position -1 after cleavage of the GST component from the desired polypeptide. Variants which result from expression in other vector systems are also contemplated.

Detail Description Paragraph - DETX (74):

[0175] In an effort to generate antibodies that recognize Atr-2, two regions of Atr-2 were expressed as GST fusion proteins. The first fusion construct encoded the entire kinase domain, a region comprised of both conserved amino acids and unique amino acids in comparison to kinase domains of other PIK-related kinases Sequences amplified in PCR using primers 22f and 15157 were ligated into the EcoRI site of pGEX-3.times. and the ligation mixture was used to transform the bacterial strain, TOP10F'. Six colonies were generated and sequence analysis of the clones revealed that the Atr-2 protein coding sequences were in-frame with GST coding sequences, suggesting that a GST-Atr-2 fusion protein should be produced from the transformed bacteria upon induction

with IPTG. Induction of these bacteria, however, did not show large amounts of GST-Atr-2 fusion protein.

Detail Description Paragraph - DETX (76):

[0177] The second GST fusion construct encoded sequences within the kinase domain of Atr-2 that are unique to Atr-2 when compared to Atr, Atm, DNA-PK, FRAP, and TRRAP. Two primers, MFA-F and TQS-R, were designed.

PGPUB-DOCUMENT-NUMBER: 20020177231

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020177231 A1

TITLE: LEUPAXIN MATERIALS AND METHODS

PUBLICATION-DATE: November 28, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
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| LIPSKY, BRIAN P. | SEATTLE | WA | US | |
| GRAY, PATRICK W. | SEATTLE | WA | US | |


APPL-NO: 09/ 211424

DATE FILED: December 15, 1998

CONTINUED PROSECUTION APPLICATION: This is a publication of a continued prosecution application (CPA) filed under 37 CFR 1.53(d).

US-CL-CURRENT: 436/6, 530/350

ABSTRACT:

Disclosed are novel leupaxin polypeptides, polynucleotides encoding the polypeptides, expression constructs comprising the polynucleotides, host cell transformed with the polynucleotides, methods to produce the polypeptides, antibodies and binding partners specific for the polypeptides, methods to identify modulators of the polypeptides, and methods to identify modulators of polypeptide expression.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (18):

[0016] The invention also embraces leupaxin variants having additional amino acid residues which result from use of specific expression systems. For example, use of commercially available vectors that express a desired polypeptide such as a glutathione-S-transferase (GST) fusion product provide the desired polypeptide having an additional glycine residue at position -1 as a result of cleavage of the GST component from the desired polypeptide. Variants which result from expression in other vector systems are also contemplated.

Detail Description Paragraph - DETX (90):

[0091] Amplification was carried out in 30 cycles of 94.degree. C. for 30 seconds, 42.degree. C. for 30 seconds, and 72.degree. C. for two minutes. The resulting amplification product was digested with EcoR1 and Xhol at restriction cites introduced by the PCR primer (underlined as set out above) and cloned into pGEX-4T-1 previously digested with the same enzymes. The resulting expression plasmid was designated pGST-LD. Sequencing confirmed that the isolated clone contained the human cDNA sequence encoding amino acids 2 through 149 of leupaxin in frame with the glutathione-S-transferase (GST) coding sequence of the vector.

Detail Description Paragraph - DETX (101):

[0098] Amplification was carried out in 30 cycles of 94.degree. C. for 30 seconds, 42.degree. C. for 30 seconds, and 72.degree. C. for two minutes. The resulting amplification product was digested with EcoRI and XhoI at restriction sites introduced by the PCR primers (underlined in the sequences set out above). The digestion product was cloned into plasmid pGEX-4T-1 previously digested with the same two enzymes to provide plasmid pGST-92-106. Sequencing confirmed that the isolated clone contained the human leupaxin cDNA sequence encoding amino acids 92 through 106 of leupaxin (SEQ ID NO: 32) in frame with the glutathione-S-transferase (GST) coding sequence of the vector.

PGPUB-DOCUMENT-NUMBER: 20020086031

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020086031 A1

TITLE: Compositions and vaccines containing antigen(s) of cryptosporidium parvum and of another pathogen

PUBLICATION-DATE: July 4, 2002

INVENTOR-INFORMATION:

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APPL-NO: 09/ 742512

DATE FILED: December 20, 2000

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60171399 19991221 US

US-CL-CURRENT: 424/190.1, 424/191.1 , 424/203.1

ABSTRACT:

Combination compositions including C. parvum antigen(s) or epitope(s) of interest with at least one other antigen or epitope of interest from a pathogen that causes enteric infection and/or symptoms and/or recombinant(s) and/or vector(s) and/or plasmid(s) expressing such antigen(s) or epitope(s) of interest and administration of such compositions such as to pregnant mammals and/or newborn or young mammals, for instance, pregnant cows and/or calves such as within the first month of birth, are disclosed and claimed.

RELATED APPLICATIONS

[0001] This application claims priority from U.S. Provisional application Serial No.60/171,399, filed Dec. 21, 1999. U.S. Ser. No. 60/171,399 and all documents cited therein ("appln cited documents") and all documents cited or referenced in the appln cited documents, are hereby incorporated herein by reference.

---------- KWIC --------

Detail Description Paragraph - DETX (87):
[0141] The sequences required to express the GST-P21 fusion protein are amplified by PCR in order to generate 2 fragments that can be cloned easily into the pBAD/HisA expression plasmid vector (Cat# V430-01 InVitrogen Corp., Carlsbad, Calif. 92008, USA). The first PCR is done using the pGEX-2TK plasmid (Cat# 27-4587-01 Amersham-Pharmacia Biotech) and the following primers:

Detail Description Paragraph - DETX (91):
[0145] This PCR generates a fragment of about 720 bp encoding the GST moiety with the addition of a NcoI restriction site at the 5' end for cloning purposes into pBAD/HisA; this modification adds a Glycine codon to the GST-P21 fusion

protein). This PCR fragment is then digested with NcoI and XhoI in order to get, after agarose gel electrophoresis and recovery with the GeneClean kit (BIO101 Inc.), the 710 bp NcoI-XhoI fragment (=fragment C).

Detail Description Paragraph - DETX (115):

[0166] A PCR is done to amplify the sequence encoding the GST protein and to add convenient restriction sites in 5' and 3' in order to subclone the PCR fragment into the final pBAD/HisA plasmid vector. The PCR uses the DNA of plasmid pGEX-2TK (Cat# 27-4587-01, Amersham-Pharmacia Biotech) as a template and the following primers:

Detail Description Paragraph - DETX (145):

[0192] Recombinant GST-fusion proteins (produced by E. coli transformed with plasmids pJCA155 or pJCA158) were affinity purified from the bacterial lysates, prepared as described in Example 8, using a glutathione-agarose (Cat# G4510, Sigma) or glutathione-Sepharose 4B (Cat# 17-0756-01, Amersham-Pharmacia Biotech). Bacterial lysates and the glutathione-agarose were incubated for 4 hours at +4.degree. C. GST-fusion proteins were then eluted from the agarose in a batch format with 10 mM reduced form glutathione (Cat# G4705, Sigma) under mild conditions (K. Johnson and D. Smith Gene. 1988. 67. 31-40). (Reference: Anonymous. GST gene fusion system: technical manual. 3.sup.rd edition. Arlington Heights, Ill.: Amersham-Pharmacia Biotech, 1997). Anyone skilled in the art can achieve scaling up of this process for purifying large quantities of GST-fusion proteins, from this disclosure and the knowledge in the art, without undue experimentation.

US-PAT-NO: 6815532

DOCUMENT-IDENTIFIER: US 6815532 B2

TITLE: ATR-2 cell cycle checkpoint

DATE-ISSUED: November 9, 2004

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
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| Loughney; Kate | Seattle | WA | N/A | N/A |
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APPL-NO: 09/ 957837

DATE FILED: September 21, 2001

PARENT-CASE:

This application is a divisional application of U.S. patent application Ser. No. 09/417,822, filed Oct. 14, 1999, now U.S. Pat. No. 6,344,549.

US-CL-CURRENT: 530/350, 536/23.2

ABSTRACT:

Polynucleotides encoding novel Atr-2 cell cycle checkpoint polypeptides are disclosed, along with expression constructs comprising the polynucleotides, host cells transformed with the expression constructs, methods to make the Atr-2 polypeptides using the host cells, Atr-2 polypeptides, and binding partners of the Atr-2 polypeptides.

3 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Detailed Description Text - DETX (44):

The invention also embraces Atr-2 variants having additional amino acid residues which result from use of specific expression systems. For example, use of commercially available vectors that express a desired polypeptide as part of glutathione-S-transferase (GST) fusion product provides the desired polypeptide having an additional glycine residue at position -1 after cleavage of the GST component from the desired polypeptide. Variants which result from expression in other vector systems are also contemplated.

Detailed Description Text - DETX (160):

In an effort to generate antibodies that recognize Atr-2, two regions of Atr-2 were expressed as GST fusion proteins. The first fusion construct encoded the entire kinase domain, a region comprised of both conserved amino acids and unique amino acids in comparison to kinase domains of other PIK-related kinases Sequences amplified in PCR using primers 22f and 15157 were ligated into the EcoRI site of pGEX-3.times. and the ligation mixture was used to transform the bacterial strain, TOP10F'. Six colonies were generated and sequence analysis of the clones revealed that the Atr-2 protein coding

sequences were in-frame with GST coding sequences, suggesting that a GST-Atr-2 fusion protein should be produced from the transformed bacteria upon induction with IPTG. Induction of these bacteria, however, did not show large amounts of GST-Atr-2 fusion protein.

Detailed Description Text - DETX (162):

The second GST fusion construct encoded sequences within the kinase domain of Atr-2 that are unique to Atr-2 when compared to Atr, Atm, DNA-PK, FRAP, and TRRAP. Two primers, MFA-F and TQS-R, were designed.

US-PAT-NO: 6790950

DOCUMENT-IDENTIFIER: US 6790950 B2

TITLE: Anti-bacterial vaccine compositions

DATE-ISSUED: September 14, 2004

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---|---|---|---|---|
| Lowery; David E. | Portage | MI | N/A | N/A |
| Fuller; Troy E. | Battle Creek | MI | N/A | N/A |
| Kennedy; Michael J. | Portage | MI | N/A | N/A |


APPL-NO: 09/ 809665

DATE FILED: March 15, 2001

PARENT-CASE:

This application is a continuation-in-part of U.S. patent application Ser. No: 09/545,199, filed Apr. 6, 2000, which claims benefit of U.S. Provisional Patent Application Serial Nos. 60/153,453, filed Sep. 10, 1999 and 60/128,689, filed Apr. 9, 1999.

US-CL-CURRENT: 536/23.7, 435/243 , 435/252.3 , 435/320.1 , 435/6 , 435/69.1 , 536/23.1

ABSTRACT:

Gram negative bacterial virulence genes are identified, thereby allowing the identification of novel anti-bacterial agents that target these virulence genes and their products, and the provision of novel gram negative bacterial mutants useful in vaccines.

6 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Detailed Description Text - DETX (21):
To simplify the protein purification process, a purification tag may be added either at the 5' or 3' end of the gene coding sequence. Commonly used purification tags include a stretch of six histidine residues (U.S. Pat. Nos. 5,284,933 and 5,310,663), a streptavidin-affinity tag described by Schmidt and Skerra, Protein Engineering, 6:109-122 (1993), a FLAG peptide [Hopp et al., Biotechnology, 6:1205-1210 (1988)], glutathione S-transferase [Smith and Johnson, Gene, 67:31-40 (1988)], and thioredoxin [LaVallie et al., Bio/Technology, 11:187-193 (1993)]. To remove these peptide or polypeptides, a proteolytic cleavage recognition site may be inserted at the fusion junction. Commonly used proteases are factor Xa, thrombin, and enterokinase.

Detailed Description Text - DETX (27):
The invention also embraces variant polypeptides having additional amino acid residues which result from use of specific expression systems. For

example, use of commercially available vectors that express a desired polypeptide as a fusion protein with glutathione-S-transferase (GST) provide the desired polypeptide having an additional glycine residue at position -1 following cleavage of the GST component from the desired polypeptide. Variants which result from expression using other vector systems are also contemplated.

US-PAT-NO: 6627732

DOCUMENT-IDENTIFIER: US 6627732 B1

TITLE: Glutathione derivatives and their dosage forms

DATE-ISSUED: September 30, 2003

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---|---|---|---|---|
| Sakon; Kiyoyuki | Hino | N/A | N/A | JP |
| Naniwa; Yoshimitsu | Hino | N/A | N/A | JP |
| Kobayashi; Mitsuru | Hino | N/A | N/A | JP |
| Miura; Daishiro | Hino | N/A | N/A | JP |
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| Imaizumi; Atsushi | Hino | N/A | N/A | JP |


APPL-NO: 09/ 673449

DATE FILED: October 16, 2000

PARENT-CASE:

This application is a 371 of PCT/JP99/02044, filed Apr. 16, 1999, which claims priority to JP 10-106359, filed Apr. 16, 1998.

FOREIGN-APPL-PRIORITY-DATA:

| COUNTRY | APPL-NO | APPL-DATE |
|---|---|---|
| JP | 10-106359 | April 16, 1998 |

PCT-DATA:
APPL-NO: PCT/JP99/02044
DATE-FILED: April 16, 1999
PUB-NO: WO99/54346
PUB-DATE: Oct 28, 1999
371-DATE:
102(E)-DATE:

US-CL-CURRENT: 530/331, 562/557 , 562/573

ABSTRACT:

The present invention provides a glutathione derivative having a dramatically enhanced hematopoiesis promoting effect in the living body represented by the formula (I): ##STR1## where A represents H or a C1-C20 acyl group; R.sub.1 represents a C1-C26 alkyl group or a C3-C26 alkenyl group; and R.sub.2 represents H, a C1-C26 alkyl group or a C3-C26 alkyl group, with the proviso that compounds are excluded in which R.sub.1 is a C1-C10 alkyl group or a C3-C10 alkenyl group, and simultaneously R.sub.2 is H, a C1-C10 alkyl group or a C3-C10 alkenyl group. The present invention also provides a salt of the glutathione derivative, or a colloidal composition that enables the safe and effective development of the effects of the glutathione derivative in the living body.

15 Claims, 14 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 14

---------- KWIC ---------

Detailed Description Text - DETX (42):

The method of alkyl or alkenyl esterification of a pharmaceutical agent such as .gamma.-glutamyl S (benzyl) cysteinyl-R(-)-phenyl-glycine (TER117) having the activity of inhibiting or binding glutathione S-transferase (GST) to a long chain alkyl or alkenyl having 12 carbons or more when they have carboxy groups, and the method of efficiently developing hematopoiesis-promoting activity in the target organ of bone marrow, or the method of enhancing safety by suppressing the development of non-selective physiological effects related to a variety of functions of GST, said methods being found in the present invention, are not limited to a glutathione derivative such as TER117 but can also be used for glutathione S-transferase-inhibiting or binding pharmaceutical substances such as ethacrynic acid having a completely different structure.

Other Reference Publication - OREF (2):

Paul J. Ciaccio et al., "Modulation of detoxification gene expression in human HT29 cells by glutathione-S-transferase inhibitors" Molecular Pharmacology (1995) vol. 48, No. 4, p. 639-647.

US-PAT-NO: 6344549

DOCUMENT-IDENTIFIER: US 6344549 B1
**See image for Certificate of Correction**

TITLE: ATR-2 cell cycle checkpoint

DATE-ISSUED: February 5, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---|---|---|---|---|
| Loughney; Kate | Seattle | WA | N/A | N/A |
| Keegan; Kathleen S. | Mercer Island | WA | N/A | N/A |

APPL-NO: 09/ 417822

DATE FILED: October 14, 1999

US-CL-CURRENT: 536/23.2, 435/320.1 , 435/325 , 435/69.1 , 536/23.1

ABSTRACT:

Polynucleotides encoding novel Atr-2 cell cycle checkpoint polypeptides are disclosed, along with expression constructs comprising the polynucleotides, host cells transformed with the expression constructs, methods to make the Atr-2 polypeptides using the host cells, Atr-2 polypeptides, and binding partners of the Atr-2 polypeptides.

10 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Brief Summary Text - BSTX (71):
The invention also embraces Atr-2 variants having additional amino acid residues which result from use of specific expression systems. For example, use of commercially available vectors that express a desired polypeptide as part of glutathione-S-transferase (GST) fusion product provides the desired polypeptide having an additional glycine residue at position -1 after cleavage of the GST component from the desired polypeptide. Variants which result from expression in other vector systems are also contemplated.

Detailed Description Text - DETX (75):
In an effort to generate antibodies that recognize Atr-2, two regions of Atr-2 were expressed as GST fusion proteins. The first fusion construct encoded the entire kinase domain, a region comprised of both conserved amino acids and unique amino acids in comparison to kinase domains of other PIK-related kinases. Sequences amplified in PCR using primers 22f and 15157 were ligated into the EcoRI site of pGEX-3X and the ligation mixture was used to transform the bacterial strain, TOP10F'. Six colonies were generated and sequence analysis of the clones revealed that the Atr-2 protein coding sequences were in-frame with GST coding sequences, suggesting that a GST-Atr-2 fusion protein should be produced from the transformed bacteria upon induction with IPTG. Induction of these bacteria, however, did not show large amounts of GST-Atr-2 fusion protein.

Detailed Description Text - DETX (77):

The second GST fusion construct encoded sequences within the kinase domain of Atr-2 that are unique to Atr-2 when compared to Atr, Atm, DNA-PK, FRAP, and TRRAP. Two primers, MFA-F and TQS-R, were designed.

US-PAT-NO: 6171839

DOCUMENT-IDENTIFIER: US 6171839 B1

TITLE: Soybean glutathione-S-transferase enzymes

DATE-ISSUED: January 9, 2001

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---|---|---|---|---|
| McGonigle; Brian | Wilmington | DE | N/A | N/A |
| O'Keefe; Daniel P. | Ridley Park | PA | N/A | N/A |


APPL-NO: 09/ 296715

DATE FILED: April 22, 1999

PARENT-CASE:

This is a division of application Ser. No. 08/924,747 filed Sep. 5, 1997, now U.S. Pat. No. 6,063,570.

US-CL-CURRENT: 435/193

ABSTRACT:

This invention relates to isolated nucleic acid fragments encoding all or a substantial portion of soybean (Glycine max) glutathione-S-transferase (GST) enzymes involved in the detoxification of xenobiotic compounds in plants and seeds; this invention further relates to isolated GST enzymes. The invention also relates to the construction of chimeric genes encoding all or a substantial portion of soybean GST enzymes, host cells transformed with those genes and methods for the recombinant production of soybean GST enzymes. Methods of constructing transgenic plants having altered levels of GST enzymes and screens for identifying soybean GST enzyme substrates and soybean GST enzyme inhibitors are also provided.

2 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Abstract Text - ABTX (1):

This invention relates to isolated nucleic acid fragments encoding all or a substantial portion of soybean (Glycine max) glutathione-S-transferase (GST) enzymes involved in the detoxification of xenobiotic compounds in plants and seeds; this invention further relates to isolated GST enzymes. The invention also relates to the construction of chimeric genes encoding all or a substantial portion of soybean GST enzymes, host cells transformed with those genes and methods for the recombinant production of soybean GST enzymes. Methods of constructing transgenic plants having altered levels of GST enzymes and screens for identifying soybean GST enzyme substrates and soybean GST enzyme inhibitors are also provided.

Brief Summary Text - BSTX (6):

GSTs have also been implicated in the detoxification of certain herbicides. Maize GSTs have been well characterized in relation to herbicide metabolism. Three genes from maize have been cloned: GST 29 (Shah et al., Plant Mol Biol 6, 203-211(1986)), GST 27 (Jepson et al., Plant Mol Biol 26:1855-1866, (1994)), GST 26 (Moore et al., Nucleic Acids Res 14:7227-7235 (1986)). These gene products form four GST isoforms: GST I (a homodimer of GST 29), GST II (a heterodimer of GST 29 and GST 27), GST III (a homodimer of GST 26), and GST IV (a homodimer of GST 27). GST 27 is highly inducible by safener compounds (Jepson (1994) supra; Holt et al., Planta 196:295-302, (1995)) and overexpression of GST 27 in tobacco confers alachlor resistance to transgenic tobacco (Jepson, personal communication). Additionally Bridges et al. (U.S. Pat. No. 5,589,614) disclose the sequence of a maize derived GST isoform II promoter useful for the expression of foreign genes in maize and wheat. In soybean, herbicide compounds conjugated to hGSH have been detected and correlated with herbicide selectivity (Frear et al., Physiol 20: 299-310 (1983); Brown et al., Pest Biochem Physiol 29:112-120, (1987)). This implies that hGSH conjugation is an important determinant in soybean herbicide selectivity although this hypothesis has not been characterized on a molecular level.

Brief Summary Text - BSTX (9):

Some efforts have been made to alter plant phenotypes by the expression of either plant or mammalian foreign GST genes or their promoters in mature plant tissue. For example, Helmer et al. (U.S. Pat. No. 5,073,677) teach the expression of a rat GST gene in tobacco under the control of a strong plant promoter. Similarly, Jepson et al. (WO 97/11189) disclose a chemically inducible maize GST promoter useful for the expression of foreign proteins in plants; Chilton et al. (EP 256223) discuss the construction of herbicide tolerant plants expressing a foreign plant GST gene; and Bieseler et al. (WO 96/23072) teach DNA encoding GSTIIIc, its recombinant production and transgenic plants containing the DNA having a herbicide-tolerant phenotype.

Brief Summary Text - BSTX (13):

In another embodiment, the instant invention relates to chimeric genes encoding soybean GST enzymes or to chimeric genes that comprise nucleic acid fragments as described above, the chimeric genes operably linked to suitable regulatory sequences, wherein expression of the chimeric genes results in altered levels of the encoded enzymes in transformed host cells.

Brief Summary Text - BSTX (16):

In an alternate embodiment, the present invention provides methods of obtaing a nucleic acid fragment encoding all or substantially all of the amino acid sequence encoding a soybean GST enzyme comprising either hybridization or primer-directed amplification methods known in the art and using the above described nucleic acid fragment. A primer-amplification-based method uses SEQ ID NOS.: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, or 27. The product of these methods is also part of the invention.

Brief Summary Text - BSTX (17):

Another embodiment of the invention includes a method for identifying a compound that inhibits the activity of a soybean GST enzyme encoded by the nucleic acid fragment and substantially similar and complementary nucleic acid fragments of SEQ ID NOS.: 1-28. The method has the steps: (a) transforming a host cell with the above described chimeric gene; (b) growing the transformed host cell under conditions that are suitable for expression of the chimeric gene wherein expression of the chimeric gene results in production of the GST enzyme; (c) optionally purifying the GST enzyme expressed by the transformed host cell; (d) contacting the GST enzyme with a chemical compound of interest; and (e) identifying the chemical compound of interest that reduces the activity

of the soybean GST enzyme relative to the activity of the soybean GST enzyme in the absence of the chemical compound of interest.

Brief Summary Text - BSTX (20):

In another embodiment, the invention provides a method for identifying a substrate for the soybean GST enzyme. The method comprises the steps of: (a) transforming a host cell with a chimeric gene comprising the nucleic acid fragment as described herein, the chimeric gene encoding a soybean GST enzyme operably linked to at least one suitable regulatory sequence; (b) growing the transformed host cell of step (a) under conditions that are suitable for expression of the chimeric gene resulting in production of the GST enzyme; (c) optionally purifying the GST enzyme expressed by the transformed host cell; (d) contacting the GST enzyme with a substrate candidate; and (e) comparing the activity of soybean GST enzyme with the activity of soybean GST enzyme that has been contacted with the substrate candidate and selecting substrate candidates that increase the activity of the sobyean GST enzyme relative to the activity of soybean GST enzyme in the absence of the substrate candidate. More preferably, step (d) of this method is carried out in the presence of at least one thiol donor. The isolated nucleic acid fragment of this method is selected from the group consisting of SEQ ID NOS.: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, and 27 and the soybean GST enzyme is selected from the group consisting of SEQ ID NOS.: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, and 28.

Brief Summary Text - BSTX (21):

Alternatively, methods are provided for identifying a soybean GST substrate candidate wherein the identification of the substrate candidate is based on a comparison of the phenotype of a host cell transformed with a chimeric gene expressing a soybean GST enzyme and contacted with a substrate candidate with the phenotype of a similarly transformed host cell grown without contact with a substrate candidate.

Brief Summary Text - BSTX (56):

The present invention provides novel GST nucleotide sequences and encoded proteins isolated from soybean. GST enzymes are known to function in the process of detoxification of a variety of xenobiotic compounds in plants, most notably, herbicides. Nucleic acid fragments encoding at least a portion of several soybean GST enzymes have been isolated and identified by comparison of random plant cDNA sequences to public databases containing nucleotide and protein sequences using the BLAST algorithms well known to those skilled in the art. The sequences of the present invention are useful in the construction of herbicide-tolerant transgenic plants, in the recombinant production of GST enzymes, in the development of screening assays to identify compounds inhibitory to the GST enzymes, and in screening assays to identify chemical substrates of the GSTs.

Brief Summary Text - BSTX (58):

"Glutathione S-Transferase" or "GST" refers to any plant-derived glutathione S-transferase (GST) enzyme capable of catalyzing the conjugation of glutathione, homoglutathione and other glutathione-like analogs via a sulfhydryl group to hydrophobic and electrophilic compounds. The term "GST" includes amino acid sequences longer or shorter than the length of natural GSTs, such as functional hybrid or partial fragments of GSTs, or their analogues. "GST" is not intended to be limited in scope on the basis of enzyme activity and may encompass amino acid sequences that possess no measurable enzyme activity but are substantially similar to those sequences known in the art to possess the above-mentioned glutathione conjugating activity.

Brief Summary Text - BSTX (59):

The term "class" or "GST class" refers to a grouping of the various GST enzymes according to amino acid identity. Currently, four classes have been identified and are referred to as "GST class I" "GST class II", "GST class III" and "GST class IV". The grouping of plant GSTs into three classes is described by Droog et al. (Plant Physiology 107:1139-1146 (1995)). All available amino acid sequences were aligned using the Wisconsin Genetics Computer Group package (Wisconsin Package Version 9.0, Genetics Computer Group (GCG), Madison, Wis.), and graphically represented on a phylogenetic tree. Three groups were identified: class one including the archetypical sequences from maize GST I (X06755) and GST III (X04375); class two including the archetypical sequence from Dianthus caryophyllus (M64628); and class three including the archetypical sequence soybean GH2/4 (M20363). Recently, Applicants have established a further subgroup of the plant GSTs known as class IV GSTs with its archetypical sequence being In2-1 (X58573).

Brief Summary Text - BSTX (65):

"Codon degeneracy" refers to divergence in the genetic code permitting variation of the nucleotide sequence without effecting the amino acid sequence of an encoded polypeptide. Accordingly, the instant invention relates to any nucleic acid fragment that encodes all or a substantial portion of the amino acid sequence encoding the GST enzymes as set forth in SEQ ID Nos: SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, and SEQ ID NO:28. The skilled artisan is well aware of the "codon-bias" exhibited by a specific host cell in usage of nucleotide codons to specify a given amino acid. Therefore, when synthesizing a gene fox improved expression in a host cell, it is desirable to design the gene such that its frequency of codon usage approaches the frequency of preferred codon usage of the host cell.

Brief Summary Text - BSTX (84):

For example, genes encoding other GST enzymes, either as cDNAs or genomic DNAs, could be isolated directly by using all or a portion of the instant nucleic acid fragments as DNA hybridization probes to screen libraries from any desired plant using methodology well known to those skilled in the art. Specific oligonucleotide probes based upon the instant nucleic acid sequences can be designed and synthesized by methods known in the art (Maniatis). Moreover, the entire sequences can be used directly to synthesize DNA probes by methods known to the skilled artisan such as random primers DNA labeling, nick translation, or end-labeling techniques, or RNA probes using available in vitro transcription systems. In addition, specific primers can be designed and used to amplify a part of or full-length of the instant sequences. The resulting amplification products can be labeled directly during amplification reactions or labeled after amplification reactions, and used as probes to isolate full length cDNA or genomic fragments under conditions of appropriate stringency.

Brief Summary Text - BSTX (89):

Any combination of any promoter and any terminator capable of inducing expression of a GST coding region may be used in the chimeric genetic sequence. Some suitable examples of promoters and terminators include those from nopaline synthase (nos), octopine synthase (ocs) and cauliflower mosaic virus (CaMV) genes. One type of efficient plant promoter that may be used is a high level plant promoter. Such promoters, in operable linkage with the genetic sequence for GST, should be capable of promoting expression of the GST such that the transformed plant is tolerant to an herbicide due to the presence of, or increased levels of, GST enzymatic activity. High level plant promoters that may be used in this invention include the promoter of the small subunit (ss) of the ribulose-1,5-bisphosphate carboxylase from example from soybean (Berry-Lowe et al., J. Molecular and App. Gen., 1:483-498 1982)), and the promoter of the

chlorophyll a/b binding protein. These two promoters are known to be light-induced in plant cells (See, for example, Genetic Engineering of Plants, an Agricultural Perspective, A. Cashmore, Plenum, N.Y. (1983), pages 29-38; Coruzzi, G. et al., The Journal of Biological Chemistry, 258:1399 (1983), and Dunsmuir, P. et al., Journal of Molecular and Applied Genetics, 2:285 (1983)).

Brief Summary Text - BSTX (92):

It may also be desirable to reduce or eliminate expression of the genes encoding the instant GST enzymes in plants. In order to accomplish this, chimeric genes designed for co-suppression of the instant GST enzymes can be constructed by linking the genes or gene fragments encoding the enzymes to plant promoter sequences. Alternatively, chimeric genes designed to express antisense RNA for all or part of the instant nucleic acid fragments can be constructed by linking the genes or gene fragment in reverse orientation to plant promoter sequences. Either the co-suppression or antisense chimeric genes could be introduced into plants via transformation wherein expression of the corresponding endogenous genes are reduced or eliminated.

Brief Summary Text - BSTX (93):

Plants transformed with the present GST genes will have a variety of phenotypes corresponding to the various properties conveyed by the GST class of proteins. Glutathione conjugation catalyzed by GSTs are known to result in sequestration and detoxification of a number of herbicides and other xenobiotics (Marrs et al., Annu. Rev. Plant Physiol. Plant Mol. Biol. 47:127-58 (1996)) and thus will be expected to produce transgenic plants with this phenotype. Other GST proteins are known to be induced by various environmental stresses such as salt stress (Roxas, et al., Stress tolerance in transgenic seedlings that overexpress glutathione S-transferase, Annual Meeting of the American Society of Plant Physiologists, (August 1997), abstract 1574, Final Program, Plant Biology and Supplement to Plant Physiology, 301), exposure to ozone (Sharma et al., Plant Physiology, 105 (4) (1994) 1089-1096), and exposure to industrial pollutants such as sulfur dioxide (Navari-Izzo et al., Plant Science 96 (1-2) (1994) 31-40). It is contemplated that transgenic plants, tolerant to a wide variety of stresses, may be produced by the present method by expressing foreign GST genes in suitable plant hosts.

Brief Summary Text - BSTX (94):

The instant GST enzymes produced in heterologous host cells, particularly in the cells of microbial hosts, can be used to prepare antibodies to the enzymes by methods well known to those skilled in the art. The antibodies are useful for detecting the enzymes in situ in cells or in vitro in cell extracts. Preferred heterologous host cells for production of the instant GST enzymes are microbial hosts. Microbial expression systems and expression vectors containing regulatory sequences that direct high level expression of foreign proteins are well known to those skilled in the art. Any of these could be used to construct chimeric genes for production of the instant GST enzymes. These chimeric genes could then be introduced into appropriate microorganisms via transformation to provide high level expression of the enzymes.

Brief Summary Text - BSTX (96):

Initiation control regions or promoters, which are useful to drive expression of the genes encoding the GST enzymes in the desired host cell, are numerous and familiar to those skilled in the art. Virtually any promoter capable of driving these genes is suitable for the present invention including but not limited to CYC1, HIS3, GAL1, GAL10, ADH1, PGK, PHO5, GAPDH, ADC1, TRP1, URA3, LEU2, ENO, TPI (useful for expression in Saccharomyces); AOX1 (useful for expression in Pichia); and lac, trp, .lambda.P.sub.L, .lambda.P.sub.R, T7, tac, and trc (useful for expression in E. coli).

Detailed Description Text - DETX (11):
The GSTa clone was isolated and cloned using primers derived from a published GST sequence, GH2/4 (Flurry et al., Physiologia Plantarum 94 (1995) 594-604) according to the following protocol.

Detailed Description Text - DETX (19):
Expression of Chimeric Genes Encoding Soybean GST Enzymes in Maize Cells (Monocotyledon)

Detailed Description Text - DETX (20):
A chimeric gene comprising a cDNA encoding a soybean GST enzyme in sense orientation can be constructed by polymerase chain reaction (PCR) of the cDNA clone using appropriate oligonucleotide primers. Cloning sites (NcoI or SmaI) can be incorporated into the oligonucleotides to provide proper orientation of the DNA fragment when inserted into the digested vector pML103 as described below. Amplification is then performed in a 100 uL volume in a standard PCR mix consisting of 0.4 mM of each oligonucleotide and 0.3 pM of target DNA in 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl.sub.2, 200 mM dGTP, 200 mM dATP, 200 mM dTTP, 200 mM dCTP and 0.025 unit DNA polymerase. Reactions are carried out in a Perkin-Elmer Cetus Thermocycler.TM. for 30 cycles comprising 1 min at 95.degree. C., 2 min at 55.degree. C. and 3 min at 72.degree. C., with a final 7 min extension at 72.degree. C. after the last cycle. The amplified DNA is then digested with restriction enzymes NcoI and SmaI and fractionated on a 0.7% low melting point agarose gel in 40 mM Tris-acetate, pH 8.5, 1 mM EDTA. The appropriate band can be excised from the gel, melted at 68.degree. C. and combined with a 4.9 kb NcoI-SmaI fragment of the plasmid pML103. Plasmid pML103 has been deposited under the terms of the Budapest Treaty with the ATCC and bears accession number ATCC 97366. The DNA segment from pML103 contains a 1.05 kb SalI-NcoI promoter fragment of the maize 27 kD zein gene and a 0.96 kb SmaI-SalI fragment from the 3' end of the maize 10 kD zein gene in the vector pGem9Zf(+) (Promega Corp., 7113 Benhart Dr., Raleigh, N.C.). Vector and insert DNA can be ligated at 15.degree. C. overnight, essentially as described (Maniatis). The ligated DNA may then be used to transform E. coli XL1-Blue (Epicurian Coli XL-1; Stratagene). Bacterial transformants can be screened by restriction enzyme digestion of plasmid DNA and limited nucleotide sequence analysis using the dideoxy chain termination method (DNA Sequencing Kit, U.S. Biochemical). The resulting plasmid construct would comprise a chimeric gene encoding, in the 5' to 3' direction, the maize 27 kD zein promoter, a cDNA fragment encoding a plant gst enzyme, and the 10 kD zein 3' region.

Detailed Description Text - DETX (31):
cDNA from fill length clones listed in Table 2 encoding the instant soybean GST enzymes were mserted into the ligation independent cloning (LIC) pET30 vector (Novagen, Inc., 597 Science Dr, Madison, Wis.) under the control of the T7 promoter, according to the manufacturer's instructions (see Novagen publications "LIC Vector Kits", publication number TB163 and U.S. Pat. No. 4,952,496). The vector was then used to transform BL21(DE3) competent E. coli hosts. Primers with a specific 3' extension designed for ligation independent cloning were designed to amplify the GST gene (Maniatis). Amplification products were gel-purified and annealed into the LIC vector after treatment with T4 DNA polymerase (Novagen). Insert-containing vectors were then used to transform NovaBlue competent E. coli cells and transformants were screened for the presence of viable inserts. Clones in the correct orientation with respect to the T7 promoter were transformed into BL21(DE3) competent cells (Novagen) and selected on LB agar plates containing 50 .mu.g/mL kanamycin. Colonies arising from this transformation were grown overnight at 37.degree. C. in Lauria Broth to OD 600=0.6 and induced with 1 mM IPTG and allowed to grow for an additional two hours. The culture was harvested, resuspended in binding buffer, lysed with a French press and cleared by centrifugation.

Other Reference Publication - OREF (9):
Michael A. Wosnick et al., Total Chemical Synthesis and Expression in Escherichia coli of a Maize Glutathione-Transferase (GST) Gene, Gene, 76, 153-160, 1989.

Other Reference Publication - OREF (11):
Dianne A.M. van der Kop et al., Isolation and Characterization of an Auxin-Inducible Glutathione S-Transferase Gene of Arabidopsis Thaliana, Plant Molecular Biology, 30, 839-844, 1996.

Other Reference Publication - OREF (13):
Dilip M. Shah et al., Structural Analysis of a Maize Gene Coding for Glutathione-S-Transferase Involved in Herbicide Detoxification, Plant Molecular Biology, 6, 203-211, 1986.

Other Reference Publication - OREF (17):
Thomas Flury et al., A 2,4-D-Inducible Glutathione S-Transferase from Soybean (Glycine Max). , Physiologia Plantarum, 94, 312-318, 1995.

Other Reference Publication - OREF (24):
Ulmasov et al., The Soybean GH2/4 Gene that encodes a glutathione S-Transferase has a promoter that is activated by a wide range of chemical agents, Plant Physiology, vol. 108, No. 3, Jul. 1, 1995 pp. 919-927.

US-PAT-NO: 6168954

DOCUMENT-IDENTIFIER: US 6168954 B1

TITLE: Soybean glutathione-S-transferase enzymes

DATE-ISSUED: January 2, 2001

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
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| O'Keefe; Daniel P. | Ridley Park | PA | N/A | N/A |


APPL-NO: 09/ 247373

DATE FILED: February 10, 1999

PARENT-CASE:

This is a continuation-in-part of Application Ser. No. 08/924,747 filed Sep. 5, 1997, now pending.

US-CL-CURRENT: 435/468, 435/15 , 435/252.3 , 435/410 , 435/471 , 435/6 , 536/23.2

ABSTRACT:

This invention relates to isolated nucleic acid fragments encoding all or a substantial portion of soybean glutathione-S-transferase (GST) enzymes involved in the detoxification of xenobiotic compounds in plants and seeds. The invention also relates to the construction of chimeric genes encoding all or a substantial portion of soybean GST enzymes, host cells transformed with those genes and methods for the recombinant production of soybean GST enzymes. Methods of constructing transgenic plants having altered levels of GST enzymes and screens for identifying soybean GST enzyme substrates and soybean GST enzyme inhibitors are also provided.

15 Claims, 0 Drawing figures

Exemplary Claim Number: 1

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Abstract Text - ABTX (1):

This invention relates to isolated nucleic acid fragments encoding all or a substantial portion of soybean glutathione-S-transferase (GST) enzymes involved in the detoxification of xenobiotic compounds in plants and seeds. The invention also relates to the construction of chimeric genes encoding all or a substantial portion of soybean GST enzymes, host cells transformed with those genes and methods for the recombinant production of soybean GST enzymes. Methods of constructing transgenic plants having altered levels of GST enzymes and screens for identifying soybean GST enzyme substrates and soybean GST enzyme inhibitors are also provided.

Brief Summary Text - BSTX (6):

GSTs have also been implicated in the detoxification of certain herbicides.

Maize GSTs have been well characterized in relation to herbicide metabolism. Three genes from maize have been cloned: GST 29 (Shah et al., Plant Mol Biol 6, 203-211(1986)), GST 27 (Jepson et al., Plant Mol Biol 26:1855-1866, (1994)), GST 26 (Moore et al., Nucleic Acids Res 14:7227-7235 (1986)). These gene products form four GST isoforms: GST I (a homodimer of GST 29), GST IV (a heterodimer of GST 29 and GST 27), GST III (a homodimer of GST 26), and GST IV (a homodimer of GST 27). GST 27 is highly inducible by safener compounds (Jepson (1994) supra; Holt et al., Planta 196:295-302, (1995)) and overexpression of GST 27 in tobacco confers alachlor resistance to transgenic tobacco (Jepson, personal communication). Additionally Bridges et al. (U.S. Pat. No. 5,589,614) disclose the sequence of a maize derived GST isoform II promoter useful for the expression of foreign genes in maize and wheat. In soybean, herbicide compounds conjugated to hGSH have been detected and correlated with herbicide selectivity (Frear et al., Physiol 20: 299-310 (1983); Brown et al., Pest Biochem Physiol 29:112-120, (1987)). This implies that hGSH conjugation is an important determinant in soybean herbicide selectivity although this hypothesis has not been characterized on a molecular level.

Brief Summary Text - BSTX (9):

Some efforts have been made to alter plant phenotypes by the expression of either plant or mammalian foreign GST genes or their promoters in mature plant tissue. For example, Helmer et al. (U.S. Pat. No. 5,073,677) teach the expression of a rat GST gene in tobacco under the control of a strong plant promoter. Similarly, Jepson et al. (WO 97/11189) disclose a chemically inducible maize GST promoter useful for the expression of foreign proteins in plants; Chilton et al. (EP 256223) discuss the construction of herbicide tolerant plants expressing a foreign plant GST gene; and Bieseler et al. (WO 96/23072) teach DNA encoding GSTIIIc, its recombinant production and transgenic plants containing the DNA having a herbicide-tolerant phenotype.

Brief Summary Text - BSTX (13):

In another embodiment, the instant invention relates to chimeric genes encoding soybean GST enzymes or to chimeric genes that comprise nucleic acid fragments as described above, the chimeric genes operably linked to suitable regulatory sequences, wherein expression of the chimeric genes results in altered levels of the encoded enzymes in transformed host cells.

Brief Summary Text - BSTX (16):

In an alternate embodiment, the present invention provides methods of obtaining a nucleic acid fragment encoding all or substantially all of the amino acid sequence encoding a soybean GST enzyme comprising either hybridization or primer-directed amplification methods known in the art and using the above described nucleic acid fragment. A primer-amplification-based method uses SEQ ID NOS.:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, and 55. The product of these methods is also part of the invention.

Brief Summary Text - BSTX (17):

Another embodiment of the invention includes a method for identifying a compound that inhibits the activity of a soybean GST enzyme encoded by the nucleic acid fragment and substantially similar and complementary nucleic acid fragments of SEQ ID NOS.:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, and 55. The method has the steps: (a) transforming a host cell with the above described chimeric gene; (b) growing the transformed host cell under conditions that are suitable for expression of the chimeric gene wherein expression of the chimeric gene results in production of the GST enzyme; (c) optionally purifying the GST enzyme expressed by the transformed host cell; (d) contacting the GST enzyme with a chemical compound

of interest; and (e) identifying the chemical compound of interest that reduces the activity of the soybean GST enzyme relative to the activity of the soybean GST enzyme in the absence of the chemical compound of interest.

Brief Summary Text - BSTX (20):

In another embodiment, the invention provides a method for identifying a substrate for the soybean GST enzyme. The method comprises the steps of: (a) transforming a host cell with a chimeric gene comprising the nucleic acid fragment as described herein, the chimeric gene encoding a soybean GST enzyme operably linked to at least one suitable regulatory sequence; (b) growing the transformed host cell of step (a) under conditions that are suitable for expression of the chimeric gene resulting in production of the GST enzyme; (c) optionally purifying the GST enzyme expressed by the transformed host cell; (d) contacting the GST enzyme with a substrate candidate; and (e) comparing the activity of soybean GST enzyme with the activity of soybean GST enzyme that has been contacted with the substrate candidate and selecting substrate candidates that increase the activity of the sobyean GST enzyme relative to the activity of soybean GST enzyme in the absence of the substrate candidate. More preferably, step (d) of this method is carried out in the presence of at least one thiol donor. The isolated nucleic acid fragment of this method is selected from the group consisting of SEQ ID NOS.:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, and 55, and the soybean GST enzyme is selected from the group consisting of SEQ ID NOS.:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, and 56.

Brief Summary Text - BSTX (21):

Alternatively, methods are provided for identifying a soybean GST substrate candidate wherein the identification of the substrate candidate is based on a comparison of the phenotype of a host cell transformed with a chimeric gene expressing a soybean GST enzyme and contacted with a substrate candidate with the phenotype of a similarly transformed host cell grown without contact with a substrate candidate.

Brief Summary Text - BSTX (81):

The present invention provides novel GST nucleotide sequences and encoded proteins isolated from soybean. GST enzymes are known to function in the process of detoxification of a variety of xenobiotic compounds in plants, most notably, herbicides. Nucleic acid fragments encoding at least a portion of several soybean GST enzymes have been isolated and identified by comparison of random plant cDNA sequences to public databases containing nucleotide and protein sequences using the BLAST algorithms well known to those skilled in the art. The sequences of the present invention are useful in the construction of herbicide-tolerant transgenic plants, in the recombinant production of GST enzymes, in the development of screening assays to identify compounds inhibitory to the GST enzymes, and in screening assays to identify chemical substrates of the GSTs.

Brief Summary Text - BSTX (82):

In the context of this disclosure, a number of terms shall be utilized. "Glutathione S-Transferase" or "GST" refers to any plant-derived glutathione S-transferase (GST) enzyme capable of catalyzing the conjugation of glutathione, homoglutathione and other glutathione-like analogs via a sulfhydryl group to hydrophobic and electrophilic compounds. The term "GST" includes amino acid sequences longer or shorter than the length of natural GSTs, such as functional hybrid or partial fragments of GSTs, or their analogues. "GST" is not intended to be limited in scope on the basis of enzyme activity and may encompass amino acid sequences that possess no measurable enzyme activity but are substantially similar to those sequences known in the

art to possess the above-mentioned glutathione conjugating activity.

Brief Summary Text - BSTX (83):
The term "class" or "GST class" refers to a grouping of the various GST enzymes according to amino acid identity. Currently, four classes have been identified and are referred to as "GST class I" "GST class II", "GST class III" and "GST class IV". The grouping of plant GSTs into three classes is described by Droog et al. (Plant Physiology 107:1139-1146 (1995)). All available amino acid sequences were aligned using the Wisconsin Genetics Computer Group package (Wisconsin Package Version 9.0, Genetics Computer Group (GCG), Madison, Wis.), and graphically represented on a phylogenetic tree. Three groups were identified: class one including the archetypical sequences from maize GST I (X06755) and GST III (X04375); class two including the archetypical sequence from Dianthus caryophyllus (M64628); and class three including the archetypical sequence soybean GH2/4 (M20363). Recently, Applicants have established a further subgroup of the plant GSTs known as class IV GSTs with its archetypical sequence being In2-1 (X58573).

Brief Summary Text - BSTX (91):
"Codon degeneracy" refers to divergence in the genetic code permitting variation of the nucleotide sequence without effecting the amino acid sequence of an encoded polypeptide. Accordingly, the instant invention relates to any nucleic acid fragment that encodes all or a substantial portion of the amino acid sequence encoding the GST enzymes as set forth in SEQ ID Nos: SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, and SEQ ID NO:56. The skilled artisan is well aware of the "codon-bias" exhibited by a specific host cell in usage of nucleotide codons to specify a given amino acid. Therefore, when synthesizing a gene for improved expression in a host cell, it is desirable to design the gene such that its frequency of codon usage approaches the frequency of preferred codon usage of the host cell.

Brief Summary Text - BSTX (110):
For example, genes encoding other GST enzymes, either as cDNAs or genomic DNAs, could be isolated directly by using all or a portion of the instant nucleic acid fragments as DNA hybridization probes to screen libraries from any desired plant using methodology well known to those skilled in the art. Specific oligonucleotide probes based upon the instant nucleic acid sequences can be designed and synthesized by methods known in the art (Maniatis). Moreover, the entire sequences can be used directly to synthesize DNA probes by methods known to the skilled artisan such as random primers DNA labeling, nick translation, or end-labeling techniques, or RNA probes using available in vitro transcription systems. In addition, specific primers can be designed and used to amplify a part of or fill-length of the instant sequences. The resulting amplification products can be labeled directly during amplification reactions or labeled after amplification reactions, and used as probes to isolate full length cDNA or genomic fragments under conditions of appropriate stringency.

Brief Summary Text - BSTX (115):
Any combination of any promoter and any terminator capable of inducing expression of a GST coding region may be used in the chimeric genetic sequence. Some suitable examples of promoters and terminators include those from nopaline synthase (nos), octopine synthase (ocs) and cauliflower mosaic virus (CaMV) genes. One type of efficient plant promoter that may be used is a high level plant promoter. Such promoters, in operable linkage with the genetic sequence for GST, should be capable of promoting expression of the GST such that the

transformed plant is tolerant to an herbicide due to the presence of, or increased levels of, GST enzymatic activity. High level plant promoters that may be used in this invention include the promoter of the small subunit (ss) of the ribulose-1,5-bisphosphate carboxylase from example from soybean (Berry-Lowe et al., J. Molecular and App. Gen., 1:483-498 1982)), and the promoter of the chlorophyll a/b binding protein. These two promoters are known to be light-induced in plant cells (See, for example, Genetic Engineering of Plants, an Agricultural Perspective, A. Cashmore, Plenum, N.Y. (1983), pages 29-38; Coruzzi, G. et al., The Journal of Biological Chemistry, 258:1399 (1983), and Dunsmuir, P. et al., Journal of Molecular and Applied Genetics, 2:285 (1983)).

Brief Summary Text - BSTX (118):

It may also be desirable to reduce or eliminate expression of the genes encoding the instant GST enzymes in plants. In order to accomplish this, chimeric genes designed for co-suppression of the instant GST enzymes can be constructed by linking the genes or gene fragments encoding the enzymes to plant promoter sequences. Alternatively, chimeric genes designed to express antisense RNA for all or part of the instant nucleic acid fragments can be constructed by linking the genes or gene fragment in reverse orientation to plant promoter sequences. Either the co-suppression or antisense chimeric genes could be introduced into plants via transformation wherein expression of the corresponding endogenous genes are reduced or eliminated.

Brief Summary Text - BSTX (119):

Plants transformed with the present GST genes will have a variety of phenotypes corresponding to the various properties conveyed by the GST class of proteins. Glutathione conjugation catalyzed by GSTs are known to result in sequestration and detoxification of a number of herbicides and other xenobiotics (Marrs et al., Annu. Rev. Plant Physiol Plant Mol. Biol 47:127-58 (1996)) and thus will be expected to produce transgenic plants with this phenotype. Other GST proteins are known to be induced by various environmental stresses such as salt stress (Roxas, et al., Stress tolerance in transgenic seedlings that overexpress glutathione S-transferase, Annual Meeting of the American Society of Plant Physiologists, (August 1997), abstract 1574, Final Program, Plant Biology and Supplement to Plant Physiology, 301), exposure to ozone (Sharma et al., Plant Physiology, 105 (4) (1994) 1089-1096), and exposure to industrial pollutants such as sulfir dioxide (Navari-Izzo et al., Plant Science 96 (1-2) (1994) 31-40). It is contemplated that transgenic plants, tolerant to a wide variety of stresses, may be produced by the present method by expressing foreign GST genes in suitable plant hosts.

Brief Summary Text - BSTX (120):

The instant GST enzymes produced in heterologous host cells, particularly in the cells of microbial hosts, can be used to prepare antibodies to the enzymes by ethods well known to those skilled in the art. The antibodies are useful for detecting the enzymes in situ in cells or in vitro in cell extracts. Preferred heterologous host cells for production of the instant GST enzymes are microbial hosts. Microbial expression systems and expression vectors containing regulatory sequences that direct high level expression of foreign proteins are well known to those skilled in the art. Any of these could be used to construct chimeric genes for production of the instant GST enzymes. These chimeric genes could then be introduced into appropriate microorganisms via transformation to provide high level expression of the enzymes.

Brief Summary Text - BSTX (122):

Initiation control regions or promoters, which are useful to drive expression of the genes encoding the GST enzymes in the desired host cell, are numerous and familiar to those skilled in the art. Virtually any promoter capable of driving these genes is suitable for the present invention including

but not limited to CYC1, HIS3, GAL1, GAL10, ADH1, PGK, PHO5, GAPDH, ADC1, TRP1, URA3, LEU2, ENO, TPI (useful for expression in Saccharomyces); AOX1 (useful for expression in Pichia); and lac, trp, .lambda.P.sub.L, .lambda.P.sub.R, T7, tac, and trc (useful for expression in E. coli).

Detailed Description Text - DETX (11):
The GSTa clone was isolated and cloned using primers derived from a published GST sequence, GH2/4 (Flurry et al., Physiologia Plantarum 94 (1995) 594-604) according to the following protocol.

Detailed Description Text - DETX (20):
Expression of Chimeric Genes Encoding Soybean GST Enzymes in Maize Cells (Monocotyledon)

Detailed Description Text - DETX (21):
A chimeric gene comprising a cDNA encoding a soybean GST enzyme in sense orientation can be constructed by polymerase chain reaction (PCR) of the eDNA clone using appropriate oligonucleotide primers. Cloning sites (NcoI or SmaI) can be incorporated into the oligonucleotides to provide proper orientation of the DNA fragment when inserted into the digested vector pML103 as described below. Amplification is then performed in a 100 .mu.L volume in a standard PCR mix consisting of 0.4 mM of each oligonucleotide and 0.3 pM of target DNA in 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl.sub.2, 200 mM dGTP, 200 mM dATP, 200 mM dTTP, 200 mM dCTP and 0.025 unit DNA polymerase. Reactions are carried out in a Perkin-Elmer Cetus Thermocycler.TM. for 30 cycles comprising 1 min at 95.degree. C., 2 min at 55.degree. C. and 3 min at 72.degree. C., with a final 7 min extension at 72.degree. C. after the last cycle. The amplified DNA is then digested with restriction enzymes NcoI and SmaI and fractionated on a 0.7% low melting point agarose gel in 40 mM Tris-acetate, pH 8.5, 1 mM EDTA. The appropriate band can be excised from the gel, melted at 68.degree. C. and combined with a 4.9 kb NcoI-SmaI fragment of the plasmid pML103. Plasmid pML103 has been deposited under the terms of the Budapest Treaty with the ATCC and bears accession number ATCC 97366. The DNA segment from pML103 contains a 1.05 kb SalI-NcoI promoter fragment of the maize 27 kD zein gene and a 0.96 kb SmaI-SalI fragment from the 3' end of the maize 10 kD zein gene in the vector pGem9Zf(+) (Promega Corp., 7113 Benhart Dr., Raleigh, N.C.). Vector and insert DNA can be ligated at 15.degree. C. overnight, essentially as described (Maniatis). The ligated DNA may then be used to transform E. coli XL1-Blue (Epicurian Coli XL-1; Stratagene). Bacterial transformants can be screened by restriction enzyme digestion of plasmid DNA and limited nucleotide sequence analysis using the dideoxy chain termination method (DNA Sequencing Kit, U.S. Biochemical). The resulting plasmid construct would comprise a chimeric gene encoding, in the 5' to 3' direction, the maize 27 kD zein promoter, a cDNA fragment encoding a plant gst enzyme, and the 10 kD zein 3' region.

Detailed Description Text - DETX (32):
cDNA from full length clones listed in Table 2 encoding the instant soybean GST enzymes were inserted into the ligation independent cloning (LIC) pET30 vector (Novagen, Inc., 597 Science Dr, Madison, Wis.) under the control of the T7 promoter, according to the manufacturer's instructions (see Novagen publications "LIC Vector Kits", publication number TB163 and U.S. Pat. No. 4,952,496). The vector was then used to transform BL21(DE3) competent E. coli hosts. Primers with a specific 3' extension designed for ligation independent cloning were designed to amplify the GST gene (Maniatis). Amplification products were gel-purified and annealed into the LIC vector after treatment with T4 DNA polymerase (Novagen). Insert-containing vectors were then used to transform NovaBlue competent E. coli cells and tansformants were screened for the presence of viable inserts. Clones in the correct orientation with respect to the T7 promoter were transformed into BL21(DE3) competent cells (Novagen)

and selected on LB agar plates containing 50 .mu.g/mL kanamycin. Colonies arising from this transformation were grown overnight at 37.degree. C. in Lauria Broth to OD 600=0.6 and induced with 1 mM IPTG and allowed to grow for an additional two hours. The culture was harvested, resuspended in binding buffer, lysed with a French press and cleared by centrifugation.

Detailed Description Paragraph Table - DETL (1):

TABLE 1 cDNA Libraries From Soybean Tissues GST Library Class Clone Tissue se1 I se1.27b04 Soybean embryo ssm II ssm.pk0026.g11 soybean shoot meristem NA III GSTa NA se3 III se3.03b09 Soybean embryo se6 III se6.pk0037.h4 Soybean embryo se6 III se6.pk0048.d7 Soybean embryo ses8w III ses8w.pk0028.c6 mature embryo 8 weeks after subculture sr1 III sr1.pk0011.d6 Soybean root library ssl III ssl.pk0002.f7 soybean seedling 5-10 day ssl III ssl.pk0005.e6 soybean seedling 5-10 day ssl III ssl.pk0014.a1 soybean seedling 5-10 day ssl III ssl.pk0020.b10 soybean seedling 5-10 day ssm III ssm.pk0067.g5 soybean shoot meristem se1 IV se1.pk0017.f5 Soybean embryo sfl1 gst I sfl1.pk126.i6 Soybean (Glycine max L.) immature flower sde4c gst I sde4c.pk002.d4 Soybean (Glycine max L.) developing embryo (9-11 mm) sdp2c gst I sdp2c.pk002.l16 Soybean (Glycine max L.) developing pods 6-7 mm sls1c gst I sls1c.pk003.f24 Soybean (Glycine max L., S1990) infected with Sclerotinia sclerotiorum mycelium sl2 gst III sl2.pk0010.e2 Soybean (Glycine max L.) two week old developing seedlings treated with 2.5 ppm chlorimuron sgs2c gst III sgs2c.pk001.n19 Soybean (Glycine max L.) seeds 14 hrs after germination sfl1 gst III sfl1.pk127.o7 Soybean (Glycine max L.) immature flower srr3c gst III srr3c.pk001.a17 Soybean (Glycine max L., Bell) roots sgs1c gst III sgs1c.pk001.c16 Soybean (Glycine max L.) seeds 4 hrs after germination sls2c gst III sls2c.pk002.d9 Soybean (Glycine max L., Manta) infected with Sclerotinia sclerotiorum mycelium sls1c gst III sls1c.pk007.i17 Soybean (Glycine max L., S1990) infected with Sclerotinia sclerotiorum mycelium src3c gst III src3c.pk026.e6 Soybean (Glycine max L., Bell) 8 day old root inoculated with eggs of Cyst Nematode (Race14) for 4 days

Claims Text - CLTX (23):

(a) transforming a host cell with a chimeric gene comprising the nucleic acid of claim 1 encoding a soybean GST enzyme, the chimeric gene operably linked to at least one suitable regulatory sequence;

Claims Text - CLTX (24):

(b) growing the transformed host cell of step (a) under conditions suitable for expression of the chimeric gene resulting in production of the GST enzyme;

Claims Text - CLTX (31):

(a) transforming a host cell with a chimeric gene comprising an isolated nucleic acid of claim 1 encoding a soybean GST enzyme, the chimeric gene operably linked to at least one suitable regulatory sequence;

Claims Text - CLTX (32):

(b) growing the transformed host cell of step (a) under conditions suitable for expression of the chimeric gene resulting in production of the soybean GST enzyme;

Other Reference Publication - OREF (5):

Ulmasov et al., The Soybean GH2/4 Gene that encodes a glutathione S-Transferase has a promoter that is activated by a wide range of chemical agents, Plant Physiology, vol. 108, No. 3, Jul. 1, 1995 pp. 919-927.

Other Reference Publication - OREF (16):

Michael A. Wosnick et al., Total Chemical Synthesis and Expression in Escherichia coli of a Maize Gluthathione-Transferase (GST) Gene, Gene, 76,

153-160, 1989.

Other Reference Publication - OREF (18):
Dianne A.M. van der Kop et al., Isolation and Characterization of an Auxin-Inducible Glutathione S-Transferase Gene of ARabidopsis Thaliana, Plant Molecular Biology, 30, 839-844, 1996.

Other Reference Publication - OREF (20):
Dilip M. Shah et al., Structural Analysis of a Maize Gene Coding for Glutathione-S-Transferase Involved in Herbicide Detoxification, Plant Molecular Biology,6, 203-211, 1986.

Other Reference Publication - OREF (23):
Thomas Flury et al., A 2,4-D-Inducible Glutathione S-Transferase from Soybean (Glycine Max)., Physiologia Plantarum, 94, 312-318, 1995.

US-PAT-NO: 6096504

DOCUMENT-IDENTIFIER: US 6096504 A

TITLE: Maize glutathione-S-transferase enzymes

DATE-ISSUED: August 1, 2000

INVENTOR-INFORMATION:

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APPL-NO: 09/ 248335

DATE FILED: February 10, 1999

PARENT-CASE:

This is a continuation-in-part of application Ser. No. 08/924,759 filed Sep. 5, 1997, now U.S. Pat. No. 5,962,229.

US-CL-CURRENT: 435/6, 435/193 , 435/252.33 , 435/320.1 , 435/410 , 435/455 , 536/23.1 , 536/23.2 , 536/23.6

ABSTRACT:

This invention relates to isolated nucleic acid fragments encoding all or a substantial portion of maize glutathione-S-transferase (GST) enzymes involved in the detoxification of xenobiotic compounds in plants and seeds. The invention also relates to the construction of chimeric genes encoding all or a substantial portion of maize GST enzymes, host cells transformed with those genes and methods of the recombinant production of maize GST enzymes. Methods of constructing transgenic plants having altered levels of GST enzymes and screens for identifying maize GST enzyme substrates and maize GST enzyme inhibitor, are also provided.

8 Claims, 0 Drawing figures

Exemplary Claim Number: 1

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This invention relates to isolated nucleic acid fragments encoding all or a substantial portion of maize glutathione-S-transferase (GST) enzymes involved in the detoxification of xenobiotic compounds in plants and seeds. The invention also relates to the construction of chimeric genes encoding all or a substantial portion of maize GST enzymes, host cells transformed with those genes and methods of the recombinant production of maize GST enzymes. Methods of constructing transgenic plants having altered levels of GST enzymes and screens for identifying maize GST enzyme substrates and maize GST enzyme inhibitor, are also provided.

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GSTs have also been implicated in the detoxification of certain herbicides.

Maize GSTs have been well characterized in relation to herbicide metabolism. Three genes from maize have been cloned: GST 29 (Shah et al., Plant Mol Biol 6, 203-211(1986)), GST 27 (Jepson et al., Plant Mol Biol 26:1855-1866, (1994)), GST 26 (Moore et al., Nucleic Acids Res 14:7227-7235 (1986)). These gene products form four GST isoforms: GST I (a homodimer of GST 29), GST II (a heterodimer of GST 29 and GST 27), GST III (a homodimer of GST 26), and GST IV (a homodimer of GST 27). GST 27 is

Brief Summary Text - BSTX (7):

highly inducible by safener compounds (Jepson (1994) supra; Holt et al., Planta 196:295-302, (1995)) and overexpression of GST 27 in tobacco confers alachlor resistance to transgenic tobacco (Jepson, personal communication). Additionally, Bridges et al. (U.S. Pat. No. 5,589,614) disclose the sequence of a maize derived GST isoform II promoter useful for the expression of foreign genes in maize and wheat. In soybean, herbicide compounds conjugated to hGSH have been detected and correlated with herbicide selectivity (Frear et al., Physiol 20: 299-310 (1983); Brown et al., Pest Biochem Physiol 29:112-120, (1987)). This implies that hGSH conjugation is an important determinant in soybean herbicide selectivity although this hypothesis has not been characterized on a molecular level.

Brief Summary Text - BSTX (8):

Some efforts have been made to alter plant phenotypes by the expression of either plant or mammalian foreign GST genes or their promoters in mature plant tissue. For example, Helmer et al. (U.S. Pat No. 5,073,677) teach the expression of a rat GST gene in tobacco under the control of a strong plant promoter. Similarly, Jepson et al. (WO 97/11189) disclose a chemically inducible maize GST promoter useful for the expression of foreign proteins in plants; Chilton et al. (EP 256223) discuss the construction of herbicide tolerant plants expressing a foreign plant GST gene; and Bieseler et al. (WO 96/23072) teach DNA encoding GSTIIIc, its recombinant production and transgenic plants containing the DNA having a herbicide-tolerant phenotype.

Brief Summary Text - BSTX (12):

In another embodiment, the instant invention relates to chimeric genes encoding maize GST enzymes or to chimeric genes that comprise nucleic acid fragments as described above, the chimeric genes operably linked to suitable regulatory sequences, wherein expression of the chimeric genes results in altered levels of the encoded enzymes in transformed host cells.

Brief Summary Text - BSTX (15):

In an alternate embodiment, the present invention provides methods of obtaining a nucleic acid fragment encoding all or substantially all of the amino acid sequence encoding a maize GST enzyme comprising either hybridization or primer-directed amplification methods known in the art and using the above described nucleic acid fragment. A primer-amplification-based method uses SEQ ID NOS.:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71 or 73. The product of these methods is also part of the invention.

Brief Summary Text - BSTX (16):

Another embodiment of the invention includes a method for identifying a compound that inhibits the activity of a maize GST enzyme encoded by the nucleic acid fragment and substantially similar and complementary nucleic acid fragments of SEQ ID NOS.:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71 and 73. The method has the steps: (a) transforming a host cell with the above described chimeric gene; (b) growing the transformed host cell under conditions that are suitable for expression of the chimeric gene wherein

expression of the chimeric gene results in production of the GST enzyme; (c) optionally purifying the GST enzyme expressed by the transformed host cell; (d) contacting the GST enzyme with a chemical compound of interest; and (e) identifying the chemical compound of interest that reduces the activity of the maize GST enzyme relative to the activity of the maize GST enzyme in the absence of the chemical compound of interest.

Brief Summary Text - BSTX (19):
In another embodiment, the invention provides a method for identifying a substrate for the maize GST enzyme. The method comprises the steps of: (a) transforming a host cell with a chimeric gene comprising the nucleic acid fragment as described herein, the chimeric gene encoding a maize GST enzyme operably linked to at least one suitable regulatory sequence; (b) growing the transformed host cell of step (a) under conditions that are suitable for expression of the chimeric gene resulting in production of the GST enzyme; (c) optionally purifying the GST enzyme expressed by the transformed host cell; (d) contacting the GST enzyme with a substrate candidate; and (e) comparing the activity of maize GST enzyme with the activity of maize GST enzyme that has been contacted with the substrate candidate and selecting substrate candidates that increase the activity of the maize GST enzyme relative to the activity of maize GST enzyme in the absence of the substrate candidate. More preferably, step (d) of this method is carried out in the presence of at least one thiol donor. The isolated nucleic acid fragment of this method is selected from the group consisting of SEQ ID NOS.:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71 and 73 and the maize GST enzyme is selected from the group consisting of SEQ ID NOS.:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, and 74.

Brief Summary Text - BSTX (20):
Alternatively, methods are provided for identifying a maize GST substrate candidate wherein the identification of the substrate candidate is based on a comparison of the phenotype of a host cell transformed with a chimeric gene expressing a maize GST enzyme and contacted with a substrate candidate with the phenotype of a similarly transformed host cell grown without contact with a substrate candidate.

Brief Summary Text - BSTX (103):
The present invention provides novel GST nucleotide sequences and encoded proteins isolated from maize. GST enzymes are known to function in the process of detoxification of a variety of xenobiotic compounds in plants, most notably, herbicides. Nucleic acid fragments encoding at least a portion of several maize GST enzymes have been isolated and identified by comparison of random plant cDNA sequences to public databases containing nucleotide and protein sequences using the BLAST algorithms well known to those skilled in the art. The sequences of the present invention are useful in the construction of herbicide-tolerant transgenic plants, in the recombinant production of GST enzymes, in the development of screening assays to identify compounds inhibitory to the GST enzymes, and in screening assays to identify chemical substrates of the GSTs.

Brief Summary Text - BSTX (105):
As use herein "Glutathione S-Transferase" or "GST" refers to any plant derived glutathione S-transferase (GST) enzyme capable of catalyzing the conjugation of glutathione, homoglutathione and other glutathione-like analogs via a sulfhydryl group, to hydrophobic and electrophilic compounds. The term GST includes amino acid sequences longer or shorter than the length of natural GSTs, such as functional hybrid or partial fragments of GSTs, or their

analogues. As used herein "GST" is not intended to be delimited on the basis of enzyme activity but may encompass amino acid sequences that possess no measurable enzyme activity but are substantially similar in to those sequences, known in the art to possess the above mentioned glutathione conjugating activity.

Brief Summary Text - BSTX (106):
The term "class" or "GST class" refers to a grouping of the various GST enzymes according to amino acid identity. Currently, four classes have been identified and are referred to as "GST class I" "GST class II", "GST class III" and "GST class IV". The grouping of plant GSTs into three classes is described by Droog et al. (Plant Physiology 107:1139-1146 (1995)). All available amino acid sequences were aligned using the Wisconsin Genetics Computer Group package (Wisconsin Package Version 9.0, Genetics Computer Group (GCG), Madison, Wis.), and graphically represented on a phylogenetic tree. Three groups were identified: class one including the archetypical sequences from maize GST I (X06755) and GST III (X04375); class two including the archetypical sequence from Dianthus caryophyllus (M64628); and class three including the archetypical sequence soybean GH2/4 (M20363). Recently, Applicants have established a further subgroup of the plant GSTs known as class IV GSTs with its archetypical sequence being In2-1 (X58573).

Brief Summary Text - BSTX (115):
"Codon degeneracy" refers to divergence in the genetic code permitting variation of the nucleotide sequence without effecting the amino acid sequence of an encoded polypeptide. Accordingly, the instant invention relates to any nucleic acid fragment that encodes all or a substantial portion of the amino acid sequence encoding the GST enzymes as set forth in SEQ ID Nos: SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72 and SEQ ID NO:74. The skilled artisan is well aware of the "codon-bias" exhibited by a specific host cell in usage of nucleotide codons to specify a given amino acid. Therefore, when synthesizing a gene for improved expression in a host cell, it is desirable to design the gene such that its frequency of codon usage approaches the frequency of preferred codon usage of the host cell.

Brief Summary Text - BSTX (136):
For example, genes encoding other GST enzymes, either as cDNAs or genomic DNAs, could be isolated directly by using all or a portion of the instant nucleic acid fragments as DNA hybridization probes to screen libraries from any desired plant using methodology well known to those skilled in the art. Specific oligonucleotide probes based upon the instant nucleic acid sequences can be designed and synthesized by methods known in the art (Maniatis). Moreover, the entire sequences can be used directly to synthesize DNA probes by methods known to the skilled artisan such as random primers DNA labeling, nick translation, or end-labeling techniques, or RNA probes using available in vitro transcription systems. In addition, specific primers can be designed and used to amplify a part of or full-length of the instant sequences. The resulting amplification products can be labeled directly during amplification reactions or labeled after amplification reactions, and used as probes to isolate full length cDNA or genomic fragments under conditions of appropriate stringency.

Brief Summary Text - BSTX (141):
Any combination of any promoter and any terminator capable of inducing

expression of a GST coding region may be used in the chimeric genetic sequence. Some suitable examples of promoters and terminators include those from nopaline synthase (nos), octopine synthase (ocs) and cauliflower mosaic virus (CaMV) genes. One type of efficient plant promoter that may be used is a high level plant promoter. Such promoters, in operable linkage with the genetic sequence for GST, should be capable of promoting expression of the GST such that the transformed plant is tolerant to an herbicide due to the presence of, or increased levels of, GST enzymatic activity. High level plant promoters that may be used in this invention include the promoter of the small subunit (ss) of the ribulose-1,5-bisphosphate carboxylase from example from soybean (Berry-Lowe et al., J. Molecular and App. Gen., 1:483-498 1982)), and the promoter of the chlorophyll a/b binding protein. These two promoters are known to be light-induced in plant cells (See, for example, Genetic Engineering of Plants, an Agricultural Perspective, A. Cashmore, Plenum, New York (1983), pages 29-38; Coruzzi, G. et al., The Journal of Biological Chemistry, 258:1399 (1983), and Dunsmuir, P. et al., Journal of Molecular and Applied Genetics, 2:285 (1983)).

Brief Summary Text - BSTX (144):

It may also be desirable to reduce or eliminate expression of the genes encoding the instant GST enzymes in plants for some applications. In order to accomplish this, chimeric genes designed for co-suppression of the instant GST enzymes can be constructed by linking the genes or gene fragments encoding the enzymes to plant promoter sequences. Alternatively, chimeric genes designed to express antisense RNA for all or part of the instant nucleic acid fragments can be constructed by linking the genes or gene fragment in reverse orientation to plant promoter sequences. Either the co-suppression or antisense chimeric genes could be introduced into plants via transformation wherein expression of the corresponding endogenous genes are reduced or eliminated.

Brief Summary Text - BSTX (145):

Plants transformed with the present GST genes will have a variety of phenotypes corresponding to the various properties conveyed by the GST class of proteins. Glutathione conjugation catalyzed by GSTs is known to result in sequestration and detoxification of a number of herbicides and other xenobiotics (Marrs et al., Annu. Rev. Plant Physiol. Plant Mol. Biol. 47:127-58 (1996)) and thus will be expected to produce transgenic plants with this phenotype. Other GST proteins are known to be induced by various environmental stresses such as salt stress (Roxas, et al., Stress tolerance in transgenic seedlings that overexpress glutathione S-transferase, Annual Meeting of the American Society of Plant Physiologists, (August 1997), abstract 1574, Final Program, Plant Biology and Supplement to Plant Physiology, 301), exposure to ozone (Sharma et al., Plant Physiology, 105 (4) (1994) 1089-1096), and exposure to industrial pollutants such as sulfur dioxide (Navari-Izzo et al., Plant Science 96 (1-2) (1994) 31-40). It is contemplated that transgenic plants, tolerant to a wide variety of stresses, may be produced by the present method by expressing foreign GST genes in suitable plant hosts.

Brief Summary Text - BSTX (146):

The instant GST enzymes produced in heterologous host cells, particularly in the cells of microbial hosts, can be used to prepare antibodies to the enzymes by methods well known to those skilled in the art. The antibodies are useful for detecting the enzymes in situ in cells or in vitro in cell extracts. Preferred heterologous host cells for production of the instant GST enzymes are microbial hosts. Microbial expression systems and expression vectors containing regulatory sequences that direct high level expression of foreign proteins are well known to those skilled in the art. Any of these could be used to construct chimeric genes for production of the instant GST enzymes. These chimeric genes could then be introduced into appropriate microorganisms via transformation to provide high level expression of the enzymes.

Brief Summary Text - BSTX (148):

Initiation control regions or promoters, which are useful to drive expression of the genes encoding the GST enzymes in the desired host cell are numerous and familiar to those skilled in the art. Virtually any promoter capable of driving these genes is suitable for the present invention including but not limited to CYC1, HIS3, GAL1, GAL10, ADH1, PGK, PHO5, GAPDH, ADC1, TRP1, URA3, LEU2, ENO, TPI (useful for expression in Saccharomyces); AOX1 (useful for expression in Pichia); and lac, trp, .lambda.P.sub.L, .lambda.P.sub.R, T7, tac, and trc (useful for expression in E. coli).

Detailed Description Text - DETX (14):

Expression of Chimeric Genes Encoding Maize GST Enzymes in Maize Cells (Monocotyledon)

Detailed Description Text - DETX (15):

A chimeric gene comprising a cDNA encoding a maize GST enzyme in sense orientation can be constructed by polymerase chain reaction (PCR) of the cDNA clone using appropriate oligonucleotide primers. Cloning sites (NcoI or SmaI) can be incorporated into the oligonucleotides to provide proper orientation of the DNA fragment when inserted into the digested vector pML103 as described below. Amplification is then performed in a 100 .mu.L volume in a standard PCR mix consisting of 0.4 mM of each oligonucleotide and 0.3 pM of target DNA in 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl.sub.2, 200 mM dGTP, 200 mM dATP, 200 mM dTTP, 200 mM dCTP and 0.025 unit DNA polymerase. Reactions are carried out in a Perkin-Elmer Cetus Thermocycler.TM. for 30 cycles comprising 1 min at 95.degree. C., 2 min at 55.degree. C. and 3 min at 72.degree. C., with a final 7 min extension at 72.degree. C. after the last cycle. The amplified DNA is then digested with restriction enzymes NcoI and SmaI and fractionated on a 0.7% low melting point agarose gel in 40 mM Tris-acetate, pH 8.5, 1 mM EDTA. The appropriate band can be excised from the gel, melted at 68.degree. C. and combined with a 4.9 kb NcoI-SmaI fragment of the plasmid pML103. Plasmid pML103 has been deposited under the terms of the Budapest Treaty at ATCC (American Type Culture Collection, 12301 Parklawn Drive, Rockville, Md. 20852), and bears accession number ATCC 97366. The DNA segment from pML103 contains a 1.05 kb SalI-NcoI promoter fragment of the maize 27 kD zein gene and a 0.96 kb SmaI-SalI fragment from the 3' end of the maize 10 kD zein gene in the vector pGem9Zf(+) (Promega Corp 7113 Benhart Dr, Raleigh, N.C.). Vector and insert DNA can be ligated at 15.degree. C. overnight, essentially as described (Maniatis). The ligated DNA may then be used to transform E. coli XL1-Blue (Epicurian Coli XL-1; Stratagene). Bacterial transformants can be screened by restriction enzyme digestion of plasmid DNA and limited nucleotide sequence analysis using the dideoxy chain termination method DNA Sequencing Kit; U.S. Biochemical). The resulting plasmid construct would comprise a chimeric gene encoding, in the 5' to 3' direction, the maize 27 kD zein promoter, a cDNA fragment encoding a plant GST enzyme, and the 10 kD zein 3' region. The chimeric gene described above can then be introduced into corn cells by the following procedure. Immature corn embryos can be dissected from developing caryopses derived from crosses of the inbred corn lines H99 and LH132 (Indiana Agric. Exp. Station, Ind., USA). The embryos are isolated 10 to 11 days after pollination when they are 1.0 to 1.5 mm long. The embryos are then placed with the axis-side facing down and in contact with agarose-solidified N6 medium (Chu et al., (1975) Sci. Sin. Peking 18:659-668). The embryos are kept in the dark at 27.degree. C. Friable embryogenic callus consisting of undifferentiated masses of cells with somatic proembryoids and embryoids borne on suspensor structures proliferates from the scutellum of these immature embryos. The embryogenic callus isolated from the primary explant can be cultured on N6 medium and sub-cultured on this medium every 2 to 3 weeks. The plasmid, p35S/Ac (obtained from Dr. Peter Eckes,

Hoechst Ag, v Frankfurt, Germany) may be used in transformation experiments in order to provide for a selectable marker. This plasmid contains the Pat gene (see European Patent Publication 0 242 236) which encodes phosphinothricin acetyl transferase (PAT). The enzyme PAT confers resistance to herbicidal glutamine synthetase inhibitors such as phosphinothricin. The pat gene in p35S/Ac is under the control of the 35S promoter from Cauliflower Mosaic Virus (Odell et al. (1985) Nature 313:810-812) and the 3M region of the nopaline synthase gene from the T-DNA of the Ti plasmid of Agrobacterium tumefaciens. The particle bombardment method (Klein et al., (1987) Nature 327:70-73) may be used to transfer genes to the callus culture cells. According to this method, gold particles ((1 .mu.m in diameter) are coated with DNA using the following technique. Ten ug of plasmid DNAs are added to 50 uL of a suspension of gold particles (60 mg per mL). Calcium chloride (50 .mu.L of a 2.5 M solution) and spermidine free base (20 .mu.L of a 1.0 M solution) are added to the particles. The suspension is vortexed during the addition of these solutions. After 10 minutes, the tubes are briefly centrifuged (5 sec at 15,000 rpm) and the supernatant removed. The particles are resuspended in 200 .mu.L of absolute ethanol, centrifuged again and the supernatant removed. The ethanol rinse is performed again and the particles resuspended in a final volume of 30 uL of ethanol. An aliquot (5 .mu.L) of the DNA-coated gold particles can be placed in the center of a flying disc (Bio-Rad Labs, 861 Ridgeview Dr, Medina, Ohio). The particles are then accelerated into the corn tissue with a PDS-1000/He (Bio-Rad Labs, 861 Ridgeview Dr, Medina, Ohio), using a helium pressure of 1000 psi, a gap distance of 0.5 cm and a flying distance of 1.0 cm. For bombardment, the embryogenic tissue is placed on filter paper over agarose-solidified N6 medium. The tissue is arranged as a thin lawn and covered a circular area of about 5 cm in diameter. The petri dish containing the tissue can be placed in the chamber of the PDS-1000/He approximately 8 cm from the stopping screen. The air in the chamber is then evacuated to a vacuum of 28 inches of Hg. The macrocarrier is accelerated with a helium shock wave using a rupture membrane that bursts when the He pressure in the shock tube reaches 1000 psi. Seven days after bombardment the tissue can be transferred to N6 medium that contains gluphosinate (2 mg per liter) and lacks casein or proline. The tissue continues to grow slowly on this medium. After an additional 2 weeks the tissue can be transferred to fresh N6 medium containing gluphosinate. After 6 weeks, areas of about 1 cm in diameter of actively growing callus can be identified on some of the plates containing the glufosinate-supplemented medium. These calli may continue to grow when sub-cultured on the selective medium. Plants can be regenerated from tie transgenic callus by first transferring clusters of tissue to N6 medium supplemented with 0.2 mg per liter of 2,4-D. After two weeks the tissue can be transferred to regeneration medium (Fromm et al., (1990) Bio/Technology 8:833-839).

Detailed Description Text - DETX (23):

cDNA from the clones bms1.pk0023.g8, cs1.pk0010.c5, ceb1.pk0017.a5, m.15.5.d06.sk20, ceb5.pk0049.a11, ceb5.pk0051.f8, and cs1.pk0059.e2, encoding the instant maize GST enzymes were inserted into the ligation independent cloning (LIC) pET30 vector (Novagen, Inc., 597 Science Dr, Madison, Wis.) under the control of the T7 promoter, according to the manufacturer's instructions (see Novagen publications "LIC Vector Kits", publication number TB163 and U.S. Pat. No. 4,952,496). The vector was then used to transform BL21(DE3) competent E. coli hosts. Primers with a specific 3' extension designed for ligation independent cloning were designed to amplify the GST gene Maniatis). Amplification products were gel-purified and annealed into the LIC vector after treatment with T4 DNA polymerase (Novagen). Insert-containing vectors were then used to transform NovaBlue competent E. coli cells and transformants were screened for the presence of viable inserts. Clones in the correct orientation with respect to the T7 promoter were transformed into BL21(DE3) competent cells

(Novagen) and selected on LB agar plates containing 50 .mu.g/mL kanamycin. Colonies arising from this transformation were grown overnight at 37.degree. C. in Lauria Broth to OD 600=0.6 and induced with 1 mM IPTG and allowed to grow for an additional two hours. The culture was harvested, resuspended in binding buffer, lysed with a French press and cleared by centrifugation.

Claims Text - CLTX (11):
(b) growing the transformed host cell produced in step (a) under conditions that are suitable for expression of the chimeric gene resulting in production of altered levels of a GST enzyme in the transformed host cell relative to expression levels of an untransformed host cell.

Claims Text - CLTX (12):
8. A method of obtaining a nucleic acid fragment encoding all or a substantial portion of the amino acid sequence encoding a GST enzyme comprising:

Claims Text - CLTX (16):
wherein the sequenced cDNA or genomic fragment encodes all or substantially all of the amino acid sequence encoding a GST enzyme.

Other Reference Publication - OREF (7):
Michael A. Wosnick et al., Total Chemical Synthesis and Expression in Escherichia coli of a Maize Glutathione-Transferase (GST) Gene, Gene, 76, 153-160, 1989.

Other Reference Publication - OREF (9):
Dianne A.M. van der Kop et al., Isolation and Characterization of an Auxin-Inducible Glutathione S-Transferase Gene of Arabidopsis Thaliana, Plant Molecular Biology, 30, 839-844, 1996.

Other Reference Publication - OREF (11):
Dilip M. Shah et al., Structural Analysis of a Maize Gene Coding for Glutathione-S-Transferase Involved in Herbicide Detoxification, Plant Molecular Biology, 6, 203-211, 1986.

Other Reference Publication - OREF (14):
Thomas Flury et al., A 2,4-D-Inducible Glutathione S-Transferase from Soybean (Glycine Max)., Physiologia Plantarum, 94, 312-318, 1995.

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DOCUMENT-IDENTIFIER: US 6063570 A

TITLE: Soybean glutathione-S-transferase enzymes

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INVENTOR-INFORMATION:

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ABSTRACT:

This invention relates to isolated nucleic acid fragments encoding all or a substantial portion of soybean glutathione-S-transferase (GST) enzymes involved in the detoxification of xenobiotic compounds in plants and seeds. The invention also relates to the construction of chimeric genes encoding all or a substantial portion of soybean GST enzymes, host cells transformed with those genes and methods for the recombinant production of soybean GST enzymes. Methods of constructing transgenic plants having altered levels of GST enzymes and screens for identifying soybean GST enzyme substrates and soybean GST enzyme inhibitors are also provided.

9 Claims, 0 Drawing figures

Exemplary Claim Number: 1

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Abstract Text - ABTX (1):

This invention relates to isolated nucleic acid fragments encoding all or a substantial portion of soybean glutathione-S-transferase (GST) enzymes involved in the detoxification of xenobiotic compounds in plants and seeds. The invention also relates to the construction of chimeric genes encoding all or a substantial portion of soybean GST enzymes, host cells transformed with those genes and methods for the recombinant production of soybean GST enzymes. Methods of constructing transgenic plants having altered levels of GST enzymes and screens for identifying soybean GST enzyme substrates and soybean GST enzyme inhibitors are also provided.

Brief Summary Text - BSTX (6):

GSTs have also been implicated in the detoxification of certain herbicides. Maize GSTs have been well characterized in relation to herbicide metabolism. Three genes from maize have been cloned: GST 29 (Shah et al., Plant Mol Biol 6, 203-211(1986)), GST 27 (Jepson et al., Plant Mol Biol 26:1855-1866, (1994)), GST 26 (Moore et al., Nucleic Acids Res 14:7227-7235 (1986)). These gene products form four GST isoforms: GST I (a homodimer of GST 29), GST II (a

heterodimer of GST 29 and GST 27), GST III (a homodimer of GST 26), and GST IV (a homodimer of GST 27). GST 27 is highly inducible by safener compounds (Jepson (1994) supra; Holt et al., Planta 196:295-302, (1995)) and overexpression of GST 27 in tobacco confers alachlor resistance to transgenic tobacco (Jepson, personal communication). Additionally Bridges et al. (U.S. Pat. No. 5,589,614) disclose the sequence of a maize derived GST isoform II promoter useful for the expression of foreign genes in maize and wheat. In soybean, herbicide compounds conjugated to hGSH have been detected and correlated with herbicide selectivity (Frear et al., Physiol 20: 299-310 (1983); Brown et al., Pest Biochem Physiol 29:112-120, (1987)). This implies that hGSH conjugation is an important determinant in soybean herbicide selectivity although this hypothesis has not been characterized on a molecular level.

Brief Summary Text - BSTX (10):

Some efforts have been made to alter plant phenotypes by the expression of either plant or mammalian foreign GST genes or their promoters in mature plant tissue. For example, Helmer et al. (U.S. Pat. No. 5,073,677) teach the expression of a rat GST gene in tobacco under the control of a strong plant promoter. Similarly, Jepson et al. (WO 97/11189) disclose a chemically inducible maize GST promoter useful for the expression of foreign proteins in plants; Chilton et al. (EP 256223) discuss the construction of herbicide tolerant plants expressing a foreign plant GST gene; and Bieseler et al. (WO 96/23072) teach DNA encoding GSTIIIc, its recombinant production and transgenic plants containing the DNA having a herbicide-tolerant phenotype.

Brief Summary Text - BSTX (14):

In another embodiment, the instant invention relates to chimeric genes encoding soybean GST enzymes or to chimeric genes that comprise nucleic acid fragments as described above, the chimeric genes operably linked to suitable regulatory sequences, wherein expression of the chimeric genes results in altered levels of the encoded enzymes in transformed host cells.

Brief Summary Text - BSTX (17):

In an alternate embodiment, the present invention provides methods of obtaining a nucleic acid fragment encoding all or substantially all of the amino acid sequence encoding a soybean GST enzyme comprising either hybridization or primer-directed amplification methods known in the art and using the above described nucleic acid fragment. A primer-amplification-based method uses SEQ ID NOS.: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, or 27. The product of these methods is also part of the invention.

Brief Summary Text - BSTX (18):

Another embodiment of the invention includes a method for identifying a compound that inhibits the activity of a soybean GST enzyme encoded by the nucleic acid fragment and substantially similar and complementary nucleic acid fragments of SEQ ID NOS.: 1-28. The method has the steps: (a) transforming a host cell with the above described chimeric gene; (b) growing the transformed host cell under conditions that are suitable for expression of the chimeric gene wherein expression of the chimeric gene results in production of the GST enzyme; (c) optionally purifying the GST enzyme expressed by the transformed host cell; (d) contacting the GST enzyme with a chemical compound of interest; and (e) identifying the chemical compound of interest that reduces the activity of the soybean GST enzyme relative to the activity of the soybean GST enzyme in the absence of the chemical compound of interest.

Brief Summary Text - BSTX (21):

In another embodiment, the invention provides a method for identifying a substrate for the soybean GST enzyme. The method comprises the steps of: (a)

transforming a host cell with a chimeric gene comprising the nucleic acid fragment as described herein, the chimeric gene encoding a soybean GST enzyme operably linked to at least one suitable regulatory sequence; (b) growing the transformed host cell of step (a) under conditions that are suitable for expression of the chimeric gene resulting in production of the GST enzyme; (c) optionally purifying the GST enzyme expressed by the transformed host cell; (d) contacting the GST enzyme with a substrate candidate; and (e) comparing the activity of soybean GST enzyme with the activity of soybean GST enzyme that has been contacted with the substrate candidate and selecting substrate candidates that increase the activity of the sobyean GST enzyme relative to the activity of soybean GST enzyme in the absence of the substrate candidate. More preferably, step (d) of this method is carried out in the presence of at least one thiol donor. The isolated nucleic acid fragment of this method is selected from the group consisting of SEQ ID NOS.: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, and 27 and the soybean GST enzyme is selected from the group consisting of SEQ ID NOS.: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, and 28.

Brief Summary Text - BSTX (22):

Alternatively, methods are provided for identifying a soybean GST substrate candidate wherein the identification of the substrate candidate is based on a comparison of the phenotype of a host cell transformed with a chimeric gene expressing a soybean GST enzyme and contacted with a substrate candidate with the phenotype of a similarly transformed host cell grown without contact with a substrate candidate.

Brief Summary Text - BSTX (57):

The present invention provides novel GST nucleotide sequences and encoded proteins isolated from soybean. GST enzymes are known to function in the process of detoxification of a variety of xenobiotic compounds in plants, most notably, herbicides. Nucleic acid fragments encoding at least a portion of several soybean GST enzymes have been isolated and identified by comparison of random plant cDNA sequences to public databases containing nucleotide and protein sequences using the BLAST algorithms well known to those skilled in the art. The sequences of the present invention are useful in the construction of herbicide-tolerant transgenic plants, in the recombinant production of GST enzymes, in the development of screening assays to identify compounds inhibitory to the GST enzymes, and in screening assays to identify chemical substrates of the GSTs.

Brief Summary Text - BSTX (58):

In the context of this disclosure, a number of terms shall be utilized. "Glutathione S-Transferase" or "GST" refers to any plant-derived glutathione S-transferase (GST) enzyme capable of catalyzing the conjugation of glutathione, homoglutathione and other glutathione-like analogs via a sulfhydryl group to hydrophobic and electrophilic compounds. The term "GST" includes amino acid sequences longer or shorter than the length of natural GSTs, such as functional hybrid or partial fragments of GSTs, or their analogues. "GST" is not intended to be limited in scope on the basis of enzyme activity and may encompass amino acid sequences that possess no measurable enzyme activity but are substantially similar to those sequences known in the art to possess the above-mentioned glutathione conjugating activity.

Brief Summary Text - BSTX (59):

The term "class" or "GST class" refers to a grouping of the various GST enzymes according to amino acid identity. Currently, four classes have been identified and are referred to as "GST class I" "GST class II", "GST class III" and "GST class IV". The grouping of plant GSTs into three classes is described by Droog et at. (Plant Physiology 107:1139-1146 (1995)). All available amino

acid sequences were aligned using the Wisconsin Genetics Computer Group package (Wisconsin Package Version 9.0, Genetics Computer Group (GCG), Madison, Wis.), and graphically represented on a phylogenetic tree. Three groups were identified: class one including the archetypical sequences from maize GST I (X06755) and GST III (X04375); class two including the archetypical sequence from Dianthus caryophyllus (M64628); and class three including the archetypical sequence soybean GH2/4 (M20363). Recently, Applicants have established a further subgroup of the plant GSTs known as class IV GSTs with its archetypical sequence being In2-1 (X58573).

Brief Summary Text - BSTX (66):

"Codon degeneracy" refers to divergence in the genetic code permitting variation of the nucleotide sequence without effecting the amino acid sequence of an encoded polypeptide. Accordingly, the instant invention relates to any nucleic acid fragment that encodes all or a substantial portion of the amino acid sequence encoding the GST enzymes as set forth in SEQ ID Nos: SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, and SEQ ID NO:28. The skilled artisan is well aware of the "codon-bias" exhibited by a specific host cell in usage of nucleotide codons to specify a given amino acid. Therefore, when synthesizing a gene for improved expression in a host cell, it is desirable to design the gene such that its frequency of codon usage approaches the frequency of preferred codon usage of the host cell.

Brief Summary Text - BSTX (86):

For example, genes encoding other GST enzymes, either as cDNAs or genomic DNAs, could be isolated directly by using all or a portion of the instant nucleic acid fragments as DNA hybridization probes to screen libraries from any desired plant using methodology well known to those skilled in the art. Specific oligonucleotide probes based upon the instant nucleic acid sequences can be designed and synthesized by methods known in the art (Maniatis). Moreover, the entire sequences can be used directly to synthesize DNA probes by methods known to the skilled artisan such as random primers DNA labeling, nick translation, or end-labeling techniques, or RNA probes using available in vitro transcription systems. In addition, specific primers can be designed and used to amplify a part of or full-length of the instant sequences. The resulting amplification products can be labeled directly during amplification reactions or labeled after amplification reactions, and used as probes to isolate full length cDNA or genomic fragments under conditions of appropriate stringency.

Brief Summary Text - BSTX (91):

Any combination of any promoter and any terminator capable of inducing expression of a GST coding region may be used in the chimeric genetic sequence. Some suitable examples of promoters and terminators include those from nopaline synthase (nos), octopine synthase (ocs) and cauliflower mosaic virus (CaMV) genes. One type of efficient plant promoter that may be used is a high level plant promoter. Such promoters, in operable linkage with the genetic sequence for GST, should be capable of promoting expression of the GST such that the transformed plant is tolerant to an herbicide due to the presence of, or increased levels of, GST enzymatic activity. High level plant promoters that may be used in this invention include the promoter of the small subunit (ss) of the ribulose-1,5-bisphosphate carboxylase from example from soybean (Berry-Lowe et at., J. Molecular and App. Gen., 1:483-498 1982)), and the promoter of the chlorophyll a/b binding protein. These two promoters are known to be light-induced in plant cells (See, for example, Genetic Engineering of Plants, an Agricultural Perspective, A. Cashmore, Plenum, New York (1983)), pages 29-38; Coruzzi, G. et al., The Journal of Biological Chemistry, 258:1399 (1983), and Dunsmuir, P. et al., Journal of Molecular and Applied Genetics,

2:285 (1983)).

Brief Summary Text - BSTX (94):
It may also be desirable to reduce or eliminate expression of the genes encoding the instant GST enzymes in plants. In order to accomplish this, chimeric genes designed for co-suppression of the instant GST enzymes can be constructed by linking the genes or gene fragments encoding the enzymes to plant promoter sequences. Alternatively, chimeric genes designed to express antisense RNA for all or part of the instant nucleic acid fragments can be constructed by linking the genes or gene fragment in reverse orientation to plant promoter sequences. Either the co-suppression or antisense chimeric genes could be introduced into plants via transformation wherein expression of the corresponding endogenous genes are reduced or eliminated.

Brief Summary Text - BSTX (95):
Plants transformed with the present GST genes will have a variety of phenotypes corresponding to the various properties conveyed by the GST class of proteins. Glutathione conjugation catalyzed by GSTs are known to result in sequestration and detoxification of a number of herbicides and other xenobiotics (Marrs et al., Annu. Rev. Plant Physiol. Plant Mol. Biol. 47:127-58 (1996)) and thus will be expected to produce transgenic plants with this phenotype. Other GST proteins are known to be induced by various environmental stresses such as salt stress (Roxas, et al., Stress tolerance in transgenic seedlings that overexpress glutathione S-transferase, Annual Meeting of the American Society of Plant Physiologists, (August 1997), abstract 1574, Final Program, Plant Biology and Supplement to Plant Physiology, 301), exposure to ozone (Sharma et al., Plant Physiology, 105 (4) (1994) 1089-1096), and exposure to industrial pollutants such as sulfur dioxide (Navari-Izzo et al., Plant Science 96 (1-2) (1994) 31-40). It is contemplated that transgenic plants, tolerant to a wide variety of stresses, may be produced by the present method by expressing foreign GST genes in suitable plant hosts.

Brief Summary Text - BSTX (96):
The instant GST enzymes produced in heterologous host cells, particularly in the cells of microbial hosts, can be used to prepare antibodies to the enzymes by methods well known to those skilled in the art. The antibodies are useful for detecting the enzymes in situ in cells or in vitro in cell extracts. Preferred heterologous host cells for production of the instant GST enzymes are microbial hosts. Microbial expression systems and expression vectors containing regulatory sequences that direct high level expression of foreign proteins are well known to those skilled in the art. Any of these could be used to construct chimeric genes for production of the instant GST enzymes. These chimeric genes could then be introduced into appropriate microorganisms via transformation to provide high level expression of the enzymes.

Brief Summary Text - BSTX (98):
Initiation control regions or promoters, which are useful to drive expression of the genes encoding the GST enzymes in the desired host cell, are numerous and familiar to those skilled in the art. Virtually any promoter capable of driving these genes is suitable for the present invention including but not limited to CYC1, HIS3, GAL1, GAL10, ADH1, PGK, PHO5, GAPDH, ADC1, TRP1, URA3, LEU2, ENO, TPI (useful for expression in Saccharomyces); AOX1 (useful for expression in Pichia); and lac, trp, .lambda.P.sub.L, .lambda.P.sub.R, T7, tac, and trc (useful for expression in E. coli).

Detailed Description Text - DETX (12):
The GSTa clone was isolated and cloned using primers derived from a published GST sequence, GH2/4 (Flurry et al., Physiologia Plantarum 94 (1995) 594-604) according to the following protocol.

Detailed Description Text - DETX (20):
Expression of Chimeric Genes Encoding Soybean GST Enzymes in Maize Cells (Monocotyledon)

Detailed Description Text - DETX (21):
A chimeric gene comprising a cDNA encoding a soybean GST enzyme in sense orientation can be constructed by polymerase chain reaction (PCR) of the cDNA clone using appropriate oligonucleotide primers. Cloning sites (NcoI or SmaI) can be incorporated into the oligonucleotides to provide proper orientation of the DNA fragment when inserted into the digested vector pML103 as described below. Amplification is then performed in a 100 uL volume in a standard PCR mix consisting of 0.4 mM of each oligonucleotide and 0.3 pM of target DNA in 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl.sub.2, 200 mM dGTP, 200 mM dATP, 200 mM dTTP, 200 mM dCTP and 0.025 unit DNA polymerase. Reactions are carried out in a Perkin-Elmer Cetus Thermocycler.TM. for 30 cycles comprising 1 min at 95.degree. C., 2 min at 55.degree. C. and 3 min at 72.degree. C., with a final 7 min extension at 72.degree. C. after the last cycle. The amplified DNA is then digested with restriction enzymes NcoI and SmaI and fractionated on a 0.7% low melting point agarose gel in 40 mM Tris-acetate, pH 8.5, 1 mM EDTA. The appropriate band can be excised from the gel, melted at 68.degree. C. and combined with a 4.9 kb NcoI-SmaI fragment of the plasmid pML103. Plasmid pML103 has been deposited under the terms of the Budapest Treaty with the ATCC and bears accession number ATCC 97366. The DNA segment from pML103 contains a 1.05 kb SalI-NcoI promoter fragment of the maize 27 kD zein gene and a 0.96 kb SmaI-SalI fragment from the 3' end of the maize 10 kD zein gene in the vector pGem9Zf(+) (Promega Corp., 7113 Benhart Dr., Raleigh, N.C.). Vector and insert DNA can be ligated at 15.degree. C. overnight, essentially as described (Maniatis). The ligated DNA may then be used to transform E. coli XL1-Blue (Epicurian Coli XL-1; Stratagene). Bacterial transformants can be screened by restriction enzyme digestion of plasmid DNA and limited nucleotide sequence analysis using the dideoxy chain termination method (DNA Sequencing Kit, U.S. Biochemical). The resulting plasmid construct would comprise a chimeric gene encoding, in the 5' to 3' direction, the maize 27 kD zein promoter, a cDNA fragment encoding a plant gst enzyme, and the 10 kD zein 3' region.

Detailed Description Text - DETX (33):
cDNA from full length clones listed in Table 2 encoding the instant soybean GST enzymes were inserted into the ligation independent cloning (LIC) pET30 vector (Novagen, Inc., 597 Science Dr, Madison, Wis.) under the control of the T7 promoter, according to the manufacturer's instructions (see Novagen publications "LIC Vector Kits", publication number TB163 and U.S. Pat. No. 4,952,496). The vector was then used to transform BL21(DE3) competent E. Coli hosts. Primers with a specific 3' extension designed for ligation independent cloning were designed to amplify the GST gene (Maniatis). Amplification products were gel-purified and annealed into the LIC vector after treatment with T4 DNA polymerase (Novagen). Insert-containing vectors were then used to transform NovaBlue competent E. coli cells and transformants were screened for the presence of viable inserts. Clones in the correct orientation with respect to the T7 promoter were transformed into BL21(DE3) competent cells (Novagen) and selected on LB agar plates containing 50 .mu.g/mL kanamycin. Colonies arising from this transformation were grown overnight at 37.degree. C. in Lauria Broth to OD 600=0.6 and induced with 1 mM IPTG and allowed to grow for an additional two hours. The culture was harvested, resuspended in binding buffer, lysed with a French press and cleared by centrifugation.

Claims Text - CLTX (12):
(b) growing the transformed host cell produced in step (a) under conditions that are suitable for expression of the chimeric gene resulting in production

of altered levels of a Glutathione S-Transferase enzyme in the transformed host cell relative to expression levels of an untransformed host cell.

Claims Text - CLTX (13):
8. A method of obtaining a nucleic acid fragment encoding all or a substantial portion of the amino acid sequence encoding a Glutathione S-Transferase enzyme comprising:

Claims Text - CLTX (18):
wherein the sequenced cDNA or genomic fragment encodes all or substantially all of the amino acid sequence encoding a Glutathione S-Transferase enzyme.

Claims Text - CLTX (19):
9. A method of obtaining a nucleic acid fragment encoding all or a substantial portion of the amino acid sequence encoding a Glutathione S-Transferase enzyme comprising:

Claims Text - CLTX (22):
wherein the amplified cDNA insert encodes a portion of an amino acid sequence encoding a Glutathione S-Transferase enzyme.

Other Reference Publication - OREF (13):
Michael A. Wosnick et al., Total Chemical Synthesis and Expression in Escherichia coli of a Maize Glutathione-Transferase (GST) Gene, Gene, 76, 153-160, 1989.

Other Reference Publication - OREF (15):
Diane A.M. van der Kop et al., Isolation and Characterization of an Auxin-Inducible Glutathione S-Transferase Gene of Arabidopsis Thaliana, Plant Molecular Biology, 30, 839-844, 1996.

Other Reference Publication - OREF (16):
Dilip M. Shah et al., Structural Analysis of a Maize Gene Coding for Glutathione-S-Transferase Involved in Herbicide Detoxification, Plant Molecular Biology, 6, 203-211, 1986.

Other Reference Publication - OREF (19):
Thomas Flury et al., A 2,4-D-Inducible Glutathione S-Transferase from Soybean (Glycine Max)., Physiologia Plantarum, 94, 312-318, 1995.

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DOCUMENT-IDENTIFIER: US 5962229 A

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INVENTOR-INFORMATION:

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ABSTRACT:

This invention relates to isolated nucleic acid fragments encoding all or a substantial portion of maize glutathione-S-transferase (GST) enzymes involved in the detoxification of xenobiotic compounds in plants and seeds. The invention also relates to the construction of chimeric genes encoding all or a substantial portion of maize GST enzymes, host cells transformed with those genes and methods of the recombinant production of maize GST enzymes. Methods of constructing transgenic plants having altered levels of GST enzymes and screens for identifying maize GST enzyme substrates and maize GST enzyme inhibitor, are also provided.

9 Claims, 0 Drawing figures

Exemplary Claim Number: 1

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Abstract Text - ABTX (1):

This invention relates to isolated nucleic acid fragments encoding all or a substantial portion of maize glutathione-S-transferase (GST) enzymes involved in the detoxification of xenobiotic compounds in plants and seeds. The invention also relates to the construction of chimeric genes encoding all or a substantial portion of maize GST enzymes, host cells transformed with those genes and methods of the recombinant production of maize GST enzymes. Methods of constructing transgenic plants having altered levels of GST enzymes and screens for identifying maize GST enzyme substrates and maize GST enzyme inhibitor, are also provided.

Brief Summary Text - BSTX (6):

GSTs have also been implicated in the detoxification of certain herbicides. Maize GSTs have been well characterized in relation to herbicide metabolism. Three genes from maize have been cloned: GST 29 (Shah et al., Plant Mol Biol 6, 203-211 (1986)), GST 27 (Jepson et al., Plant Mol Biol 26:1855-1866, (1994)), GST 26 (Moore et al., Nucleic Acids Res 14:7227-7235 (1986)). These gene products form four GST isoforms: GST I (a homodimer of GST 29), GST II (a

heterodimer of GST 29 and GST 27), GST III (a homodimer of GST 26), and GST IV (a homodimer of GST 27). GST 27 is highly inducible by safener compounds (Jepson (1994) supra; Holt et al., Planta 196:295-302, (1995)) and overexpression of GST 27 in tobacco confers alachlor resistance to transgenic tobacco (Jepson, personal communication). Additionally, Bridges et al. (U.S. Pat. No. 5,589,614) disclose the sequence of a maize derived GST isoform II promoter useful for the expression of foreign genes in maize and wheat. In soybean, herbicide compounds conjugated to hGSH have been detected and correlated with herbicide selectivity (Frear et al., Physiol 20: 299-310 (1983); Brown et al., Pest Biochem Physiol 29:112-120, (1987)). This implies that hGSH conjugation is an important determinant in soybean herbicide selectivity although this hypothesis has not been characterized on a molecular level.

Brief Summary Text - BSTX (7):

Some efforts have been made to alter plant phenotypes by the expression of either plant or mammalian foreign GST genes or their promoters in mature plant tissue. For example, Helmer et al. (U.S. Pat. No. 5,073,677) teach the expression of a rat GST gene in tobacco under the control of a strong plant promoter. Similarly, Jepson et al. (WO 97/11189) disclose a chemically inducible maize GST promoter useful for the expression of foreign proteins in plants; Chilton et al. (EP 256223) discuss the construction of herbicide tolerant plants expressing a foreign plant GST gene; and Bieseler et al. (WO 96/23072) teach DNA encoding GSTIIIc, its recombinant production and transgenic plants containing the DNA having a herbicide-tolerant phenotype.

Brief Summary Text - BSTX (11):

In another embodiment, the instant invention relates to chimeric genes encoding maize GST enzymes or to chimeric genes that comprise nucleic acid fragments as described above, the chimeric genes operably linked to suitable regulatory sequences, wherein expression of the chimeric genes results in altered levels of the encoded enzymes in transformed host cells.

Brief Summary Text - BSTX (14):

In an alternate embodiment, the present invention provides methods of obtaining a nucleic acid fragment encoding all or substantially all of the amino acid sequence encoding a maize GST enzyme comprising either hybridization or primer-directed amplification methods known in the art and using the above described nucleic acid fragment. A primer-amplification-based method uses SEQ ID NOS.: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, or 23. The product of these methods is also part of the invention.

Brief Summary Text - BSTX (15):

Another embodiment of the invention includes a method for identifying a compound that inhibits the activity of a maize GST enzyme encoded by the nucleic acid fragment and substantially similar and complementary nucleic acid fragments of SEQ ID NOS.:1-24. The method has the steps: (a) transforming a host cell with the above described chimeric gene; (b) growing the transformed host cell under conditions that are suitable for expression of the chimeric gene wherein expression of the chimeric gene results in production of the GST enzyme; (c) optionally purifying the GST enzyme expressed by the transformed host cell; (d) contacting the GST enzyme with a chemical compound of interest; and (e) identifying the chemical compound of interest that reduces the activity of the maize GST enzyme relative to the activity of the maize GST enzyme in the absence of the chemical compound of interest.

Brief Summary Text - BSTX (18):

In another embodiment, the invention provides a method for identifying a substrate for the maize GST enzyme. The method comprises the steps of: (a)

transforming a host cell with a chimeric gene comprising the nucleic acid fragment as described herein, the chimeric gene encoding a maize GST enzyme operably linked to at least one suitable regulatory sequence; (b) growing the transformed host cell of step (a) under conditions that are suitable for expression of the chimeric gene resulting in production of the GST enzyme; (c) optionally purifying the GST enzyme expressed by the transformed host cell; (d) contacting the GST enzyme with a substrate candidate; and (e) comparing the activity of maize GST enzyme with the activity of maize GST enzyme that has been contacted with the substrate candidate and selecting substrate candidates that increase the activity of the maize GST enzyme relative to the activity of maize GST enzyme in the absence of the substrate candidate. More preferably, step (d) of this method is carried out in the presence of at least one thiol donor. The isolated nucleic acid fragment of this method is selected from the group consisting of SEQ ID NOS.: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, and 23, 25 and the maize GST enzyme is selected from the group consisting of SEQ ID NOS.: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and 24.

Brief Summary Text - BSTX (19):
Alternatively, methods are provided for identifying a maize GST substrate candidate wherein the identification of the substrate candidate is based on a comparison of the phenotype of a host cell transformed with a chimeric gene expressing a maize GST enzyme and contacted with a substrate candidate with the phenotype of a similarly transformed host cell grown without contact with a substrate candidate.

Brief Summary Text - BSTX (49):
The present invention provides novel GST nucleotide sequences and encoded proteins isolated from maize. GST enzymes are known to function in the process of detoxification of a variety of xenobiotic compounds in plants, most notably, herbicides. Nucleic acid fragments encoding at least a portion of several maize GST enzymes have been isolated and identified by comparison of random plant cDNA sequences to public databases containing nucleotide and protein sequences using the BLAST algorithms well known to those skilled in the art. The sequences of the present invention are useful in the construction of herbicide-tolerant transgenic plants, in the recombinant production of GST enzymes, in the development of screening assays to identify compounds inhibitory to the GST enzymes, and in screening assays to identify chemical substrates of the GSTs.

Brief Summary Text - BSTX (51):
As use herein "Glutathione S-Transferase" or "GST" refers to any plant derived glutathione S-transferase (GST) enzyme capable of catalyzing the conjugation of glutathione, homoglutathione and other glutathione-like analogs via a sulfhydryl group, to hydrophobic and electrophilic compounds. The term GST includes amino acid sequences longer or shorter than the length of natural GSTs, such as functional hybrid or partial fragments of GSTs, or their analogues. As used herein "GST" is not intended to be delimited on the basis of enzyme activity but may encompass amino acid sequences that possess no measurable enzyme activity but are substantially similar in to those sequences, known in the art to possess the above mentioned glutathione conjugating activity.

Brief Summary Text - BSTX (52):
The term "class" or "GST class" refers to a grouping of the various GST enzymes according to amino acid identity. Currently, four classes have been identified and are referred to as "GST class I" "GST class II", "GST class III" and "GST class IV". The grouping of plant GSTs into three classes is described by Droog et al. (Plant Physiology 107:1139-1146 (1995)). All available amino acid sequences were aligned using the Wisconsin Genetics Computer Group package

(Wisconsin Package Version 9.0, Genetics Computer Group (GCG), Madison, Wis.), and graphically represented on a phylogenetic tree. Three groups were identified: class one including the archetypical sequences from maize GST I (X06755) and GST III (X04375); class two including the archetypical sequence from Dianthus caryophyllus (M64628); and class three including the archetypical sequence soybean GH2/4 (M20363). Recently, Applicants have established a further subgroup of the plant GSTs known as class IV GSTs with its archetypical sequence being In2-1 (X58573).

Brief Summary Text - BSTX (58):

"Codon degeneracy" refers to divergence in the genetic code permitting variation of the nucleotide sequence without effecting the amino acid sequence of an encoded polypeptide. Accordingly, the instant invention relates to any nucleic acid fragment that encodes all or a substantial portion of the amino acid sequence encoding the GST enzymes as set forth in SEQ ID Nos: SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, and SEQ ID NO:24. The skilled artisan is well aware of the "codon-bias" exhibited by a specific host cell in usage of nucleotide codons to specify a given amino acid. Therefore, when synthesizing a gene for improved expression in a host cell, it is desirable to design the gene such that its frequency of codon usage approaches the frequency of preferred codon usage of the host cell.

Brief Summary Text - BSTX (79):

For example, genes encoding other GST enzymes, either as cDNAs or genomic DNAs, could be isolated directly by using all or a portion of the instant nucleic acid fragments as DNA hybridization probes to screen libraries from any desired plant using methodology well known to those skilled in the art. Specific oligonucleotide probes based upon the instant nucleic acid sequences can be designed and synthesized by methods known in the art (Maniatis). Moreover, the entire sequences can be used directly to synthesize DNA probes by methods known to the skilled artisan such as random primers DNA labeling, nick translation, or end-labeling techniques, or RNA probes using available in vitro transcription systems. In addition, specific primers can be designed and used to amplify a part of or full-length of the instant sequences. The resulting amplification products can be labeled directly during amplification reactions or labeled after amplification reactions, and used as probes to isolate full length cDNA or genomic fragments under conditions of appropriate stringency.

Brief Summary Text - BSTX (84):

Any combination of any promoter and any terminator capable of inducing expression of a GST coding region may be used in the chimeric genetic sequence. Some suitable examples of promoters and terminators include those from nopaline synthase (nos), octopine synthase (ocs) and cauliflower mosaic virus (CaMV) genes. One type of efficient plant promoter that may be used is a high level plant promoter. Such promoters, in operable linkage with the genetic sequence for GST, should be capable of promoting expression of the GST such that the transformed plant is tolerant to an herbicide due to the presence of, or increased levels of, GST enzymatic activity. High level plant promoters that may be used in this invention include the promoter of the small subunit (ss) of the ribulose-1,5-bisphosphate carboxylase from example from soybean (Berry-Lowe et al., J. Molecular and App. Gen., 1:483-498 1982)), and the promoter of the chlorophyll a/b binding protein. These two promoters are known to be light-induced in plant cells (See, for example, Genetic Engineering of Plants, an Agricultural Perspective A. Cashmore, Plenum, New York (1983), pages 29-38; Coruzzi, G. et al., The Journal of Biological Chemistry, 258:1399 (1983), and Dunsmuir, P. et al., Journal of Molecular and Applied Genetics, 2:285 (1983)).

Brief Summary Text - BSTX (87):

It may also be desirable to reduce or eliminate expression of the genes encoding the instant GST enzymes in plants for some applications. In order to accomplish this, chimeric genes designed for co-suppression of the instant GST enzymes can be constructed by linking the genes or gene fragments encoding the enzymes to plant promoter sequences. Alternatively, chimeric genes designed to express antisense RNA for all or part of the instant nucleic acid fragments can be constructed by linking the genes or gene fragment in reverse orientation to plant promoter sequences. Either the co-suppression or antisense chimeric genes could be introduced into plants via transformation wherein expression of the corresponding endogenous genes are reduced or eliminated.

Brief Summary Text - BSTX (88):

Plants transformed with the present GST genes will have a variety of phenotypes corresponding to the various properties conveyed by the GST class of proteins. Glutathione conjugation catalyzed by GSTs is known to result in sequestration and detoxification of a number of herbicides and other xenobiotics (Marrs et al., Annu. Rev. Plant Physiol. Plant Mol. Biol. 47:127-58 (1996)) and thus will be expected to produce transgenic plants with this phenotype. Other GST proteins are known to be induced by various environmental stresses such as salt stress (Roxas, et al., Stress tolerance in transgenic seedlings that overexpress glutathione S-transferase, Annual Meeting of the American Society of Plant Physiologists, (August 1997), abstract 1574, Final Program, Plant Biology and Supplement to Plant Physiology, 301), exposure to ozone (Sharma et al., Plant Physiology, 105 (4) (1994)1089-1096), and exposure to industrial pollutants such as sulfur dioxide (Navari-Izo et al., Plant Science 96 (1-2) (1994) 31-40). It is contemplated that transgenic plants, tolerant to a wide variety of stresses, may be produced by the present method by expressing foreign GST genes in suitable plant hosts.

Brief Summary Text - BSTX (89):

The instant GST enzymes produced in heterologous host cells, particularly in the cells of microbial hosts, can be used to prepare antibodies to the enzymes by methods well known to those skilled in the art. The antibodies are useful for detecting the enzymes in situ in cells or in vitro in cell extracts. Preferred heterologous host cells for production of the instant GST enzymes are microbial hosts. Microbial expression systems and expression vectors containing regulatory sequences that direct high level expression of foreign proteins are well known to those skilled in the art. Any of these could be used to construct chimeric genes for production of the instant GST enzymes. These chimeric genes could then be introduced into appropriate microorganisms via transformation to provide high level expression of the enzymes.

Brief Summary Text - BSTX (91):

Initiation control regions or promoters, which are useful to drive expression of the genes encoding the GST enzymes in the desired host cell are numerous and familiar to those skilled in the art. Virtually any promoter capable of driving these genes is suitable for the present invention including but not limited to CYC1, HIS3, GAL1, GAL10, ADH1, PGK, PHO5, GAPDH, ADC1, TRP1, URA3, LEU2, ENO, TPI (useful for expression in Saccharomyces); AOX1 (useful for expression in Pichia); and lac, trp, .lambda.P.sub.L, .lambda.P.sub.R, T7, tac, and trc (useful for expression in E. coli).

Detailed Description Text - DETX (14):

Expression of Chimeric Genes Encoding Maize GST Enzymes in Maize Cells (Monocotyledon)

Detailed Description Text - DETX (15):

A chimeric gene comprising a cDNA encoding a maize GST enzyme in sense orientation can be constructed by polymerase chain reaction (PCR) of the cDNA

clone using appropriate oligonucleotide primers. Cloning sites (NcoI or SmaI) can be incorporated into the oligonucleotides to provide proper orientation of the DNA fragment when inserted into the digested vector pML103 as described below. Amplification is then performed in a 100 .mu.L volume in a standard PCR mix consisting of 0.4 mM of each oligonucleotide and 0.3 pM of target DNA in 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl.sub.2, 200 mM dGTP, 200 mM dATP, 200 mM dTTP, 200 mM dCTP and 0.025 unit DNA polymerase. Reactions are carried out in a Perkin-Elmer Cetus Thermocycler.TM. for 30 cycles comprising 1 min at 95.degree. C., 2 min at 55.degree. C. and 3 min at 72.degree. C., with a final 7 min extension at 72.degree. C. after the last cycle. The amplified DNA is then digested with restriction enzymes NcoI and SmaI and fractionated on a 0.7% low melting point agarose gel in 40 mM Tris-acetate, pH 8.5, 1 mM EDTA. The appropriate band can be excised from the gel, melted at 68.degree. C. and combined with a 4.9 kb NcoI-SmaI fragment of the plasmid pML 103. Plasmid pML 103 has been deposited under the terms of the Budapest Treaty at ATCC (American Type Culture Collection, 12301 Parklawn Drive, Rockville, Md. 20852), and bears accession number ATCC 97366. The DNA segment from pML103 contains a 1.05 kb SalI-NcoI promoter fragment of the maize 27 kD zein gene and a 0.96 kb SmaI-SalI fragment from the 3' end of the maize 10 kD zein gene in the vector pGem9Zf(+) (Promega Corp 7113 Benhart Dr, Raleigh, N.C.). Vector and insert DNA can be ligated at 15.degree. C. overnight, essentially as described (Maniatis). The ligated DNA may then be used to transform E. coli XL1-Blue (Epicurian Coli XL-1; Stratagene). Bacterial transformants can be screened by restriction enzyme digestion of plasmid DNA and limited nucleotide sequence analysis using the dideoxy chain termination method DNA Sequencing Kit; U.S. Biochemical). The resulting plasmid construct would comprise a chimeric gene encoding, in the 5' to 3' direction, the maize 27 kD zein promoter, a cDNA fragment encoding a plant GST enzyme, and the 10 kD zein 3' region. The chimeric gene described above can then be introduced into corn cells by the following procedure. Immature corn embryos can be dissected from developing caryopses derived from crosses of the inbred corn lines H99 and LH132 (Indiana Agric. Exp. Station, Ind., USA). The embryos are isolated 10 to 11 days after pollination when they are 1.0 to 1.5 mm long. The embryos are then placed with the axis-side facing down and in contact with agarose-solidified N6 medium (Chu et al., (1975) Sci. Sin. Peking 18:659-668). The embryos are kept in the dark at 27.degree. C. Friable embryogenic callus consisting of undifferentiated masses of cells with somatic proembryoids and embryoids borne on suspensor structures proliferates from the scutellum of these immature embryos. The embryogenic callus isolated from the primary explant can be cultured on N6 medium and sub-cultured on this medium every 2 to 3 weeks. The plasmid, p35S/Ac (obtained from Dr. Peter Eckes, Hoechst Ag, v Frankfurt, Germany) may be used in transformation experiments in order to provide for a selectable marker. This plasmid contains the Pat gene (see European Patent Publication 0 242 236) which encodes phosphinothricin acetyl transferase (PAT). The enzyme PAT confers resistance to herbicidal glutamine synthetase inhibitors such as phosphinothricin. The pat gene in p35S/Ac is under the control of the 35S promoter from Cauliflower Mosaic Virus (Odell et al. (1985) Nature 313:810-812) and the 3M region of the nopaline synthase gene from the T-DNA of the Ti plasmid of Agrobacterium tumefaciens. The particle bombardment method (Klein et al., (1987) Nature 327:70-73) may be used to transfer genes to the callus culture cells. According to this method, gold particles ((1 .mu.m in diameter) are coated with DNA using the following technique. Ten ug of plasmid DNAs are added to 50 uL of a suspension of gold particles (60 mg per mL). Calcium chloride (50 .mu.L of a 2.5 M solution) and spermidine free base (20 .mu.L of a 1.0 M solution) are added to the particles. The suspension is vortexed during the addition of these solutions. After 10 minutes, the tubes are briefly centrifuged (5 sec at 15,000 rpm) and the supernatant removed. The particles are resuspended in 200 .mu.L of absolute ethanol, centrifuged again and the supernatant removed. The ethanol rinse is performed again and the

particles resuspended in a final volume of 30 uL of ethanol. An aliquot (5 .mu.L) of the DNA-coated gold particles can be placed in the center of a flying disc (Bio-Rad Labs, 861 Ridgeview Dr, Medina, Ohio). The particles are then accelerated into the corn tissue with a PDS- 1000/He (Bio-Rad Labs, 861 Ridgeview Dr, Medina, Ohio), using a helium pressure of 1000 psi, a gap distance of 0.5 cm and a flying distance of 1.0 cm. For bombardment, the embryogenic tissue is placed on filter paper over agarose-solidified N6 medium. The tissue is arranged as a thin lawn and covered a circular area of about 5 cm in diameter. The petri dish containing the tissue can be placed in the chamber of the PDS-1000/He approximately 8 cm from the stopping screen. The air in the chamber is then evacuated to a vacuum of 28 inches of Hg. The macrocarrier is accelerated with a helium shock wave using a rupture membrane that bursts when the He pressure in the shock tube reaches 1000 psi. Seven days after bombardment the tissue can be transferred to N6 medium that contains gluphosinate (2 mg per liter) and lacks casein or proline. The tissue continues to grow slowly on this medium. After an additional 2 weeks the tissue can be transferred to fresh N6 medium containing gluphosinate. After 6 weeks, areas of about 1 cm in diameter of actively growing callus can be identified on some of the plates containing the glufosinate-supplemented medium. These calli may continue to grow when sub-cultured on the selective medium. Plants can be regenerated from the transgenic callus by first transferring clusters of tissue to N6 medium supplemented with 0.2 mg per liter of 2,4-D. After two weeks the tissue can be transferred to regeneration medium (Fromm et al., (1990) Bio/Technology 8:833-839).

Detailed Description Text - DETX (23):

cDNA from the clones bms1.pk0023.g8, cs1.pk0010.c5, ceb1.pk0017.a5, m.15.5.d06.sk20, ceb5.pk0049.a11, ceb5.pk0051.f8, and cs1.pk0059.e2, encoding the instant maize GST enzymes were inserted into the ligation independent cloning (LIC) pET30 vector (Novagen, Inc., 597 Science Dr, Madison, Wis.) under the control of the T7 promoter, according to the manufacturer's instructions (see Novagen publications "LIC Vector Kits", publication number TB163 and U.S. Pat. No. 4,952,496). The vector was then used to transform BL21 (DE3) competent E. coli hosts. Primers with a specific 3' extension designed for ligation independent cloning were designed to amplify the GST gene (Maniatis). Amplification products were gel-purified and annealed into the LIC vector after treatment with T4 DNA polymerase (Novagen). Insert-containing vectors were then used to transform NovaBlue competent E. coli cells and transformants were screened for the presence of viable inserts. Clones in the correct orientation with respect to the T7 promoter were transformed into BL21 (DE3) competent cells (Novagen) and selected on LB agar plates containing 50 .mu.g/mL kanamycin. Colonies arising from this transformation were grown overnight at 37.degree. C. in Lauria Broth to OD 600=0.6 and induced with 1 mM IPTG and allowed to grow for an additional two hours. The culture was harvested, resuspended in binding buffer, lysed with a French press and cleared by centrifugation.

Detailed Description Paragraph Table - DETL (2):

TABLE 2

| BLAST Results For Clones | SEQ ID NO | GST Blast | pLog | Clone Class | Similarity Identified | Base | Peptide | Algorithm | Score |
|---|---|---|---|---|---|---|---|---|---|
| bms1.pk0023.g8 | 1 | X79515.vertline.ZMGST27 Z.mays | 1 | 2 | Nnr | 122.086 | | | GST-27 mRNA for glutathione-S- transferase |
| cs1.pk0010.c5 | 1 | D17673.vertline.ATHERD13 | 3 | 4 | Nnr | 8.16 | | | Arabidopsis thaliana mRNA for glutathione S-transferase |
| ceb1.pk0017.a5 | 1 | X78203.vertline.HMGST H.muticus | 5 | 6 | Nnr | 21.51 | | | mRNA for glutathione S-transferase |
| cc71se-a.pk0001.g2 | III | (AF004358) glutathione S- | 7 | 8 | Nnr | 16.48 | | | transferase TSI-1 (Aegilops squarrosa) |
| cc71se-b.pk0014.b8 | III | | | | | | | | |

D10861.vertline.RICORFC Rice 9 10 Nnr 14.96 mRNA for a protein related to chilling tolerance. ceb5.pk0051.f8 III D10861.vertline.RICORFC Rice 11 12 Nnr 40.44 mRNA for a protein related to chilling tolerance. cr1n.pk0003.b1 III U80615.vertline.EGU80615 Eucalyptus 13 14 Nnr 24.70 globulus auxin-induced protein (EgPar) mRNA, complete cds cr1n.pk0014.g8 III M16901.vertline.MZEGSTIB Maize 15 16 Nnr 5.85 glutathione S-transferase (GST-I) mRNA, complete cds m.15.5.d06.sk20 II .vertline.M97702.vertline.DROGLUSTD 17 18 Nnr 3.63 Drosophila melanogaster glutathione S-transferase gene. cr1n.pk0040.e12 II 167970 (L05915) (GST1) gene 19 20 Xnr 42.03 product (Dianthus caryophyllus) ceb5.pk0049.a11 III .vertline.Y12862.vertline.ZYMY12862 Zea 21 22 Nnr 0.0 Maize mRNA for glutathione S- transferase cs1.pk0059.e2 III D10861.vertline.RICORFC Rice 24 25 Nnr 41.03 mRNA for a protein related to chilling tolerance.

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Claims Text - CLTX (12):
(b) growing the transformed host cell produced in step (a) under conditions that are suitable for expression of the chimeric gene resulting in production of altered levels of a Glutathione S-Transferase enzyme in the transformed host cell relative to expression levels of an untransformed host cell.

Claims Text - CLTX (13):
8. A method of obtaining a nucleic acid fragment encoding all or a substantial portion of the amino acid sequence encoding a Glutathione S-Transferase enzyme comprising:

Claims Text - CLTX (17):
wherein the sequenced cNDA or genomic fragment encodes all or substantially all of the amino acid sequence encoding a Glutathione S-Transferase enzyme.

Claims Text - CLTX (18):
9. A method of obtaining a nucleic acid fragment encoding all or a substantial portion of the amino acid sequence encoding a Glutathione S-Transferase enzyme comprising:

Claims Text - CLTX (21):
wherein the amplified cDNA insert encodes a portion of an amino acid sequence encoding a Glutathione S-Transferase enzyme.

Other Reference Publication - OREF (2):
Itzhaki et al. "Characterization of an ethylene-responsive glutathione S-transferase gene cluster in Carnation" Plant Mol. Biol. 22, 43-58, 1993.

Other Reference Publication - OREF (11):
Michael A. Wosnick et al., Total Chemical Synthesis and Expression in Escherichia coli of a Maize Glutathione-Transferase (GST) Gene, Gene, 76, 153-160, 1989.

Other Reference Publication - OREF (13):
Dianne A.M. van der Kop et al., Isolation and Characterization of an Auxin-Inducible Glutathione S-Transferase Gene of Arabidopsis Thaliana, Plant Molecular Biology, 30, 839-844, 1996.

Other Reference Publication - OREF (14):
Dilip M. Shah et al., Structural Analysis of a Maize Gene Coding for Glutathione-S-Transferase Involved in Herbicide Detoxification, Plant Molecular Biology, 6, 203-211, 1986.

Other Reference Publication - OREF (17):

Thomas Flury et al., A 2,4-D-Inducible Glutathione S-Transferase from Soybean (Glycine Max)., Physiologia Plantarum, 94, 312-318, 1995.

US-PAT-NO: 5856161

DOCUMENT-IDENTIFIER: US 5856161 A
**See image for Certificate of Correction**

TITLE: Tumor necrosis factor receptor-I-associated protein kinase and methods for its use

DATE-ISSUED: January 5, 1999

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
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APPL-NO: 08/ 580988

DATE FILED: January 3, 1996

PARENT-CASE:

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation in part of U.S. Ser. No. 08/445,410, filed May 19, 1995, now abandoned, which is a continuation in part of U.S. Ser. No. 08/271,424, filed Jul. 7, 1994, now abandoned.

US-CL-CURRENT: 435/195, 530/350 , 530/351

ABSTRACT:

The present invention provides an isolated and purified protein that associates with the cytoplasmic domain of the p60 form of the tumor necrosis factor receptor, having a molecular weight of about 52-55 kDa on SDS-PAGE, is a phosphoprotein, and does not bind to the p80 form of the tumor necrosis factor receptor. Also provided is an isolated and purified protein kinase that binds to the cytoplasmic domain of the p60 form of the tumor necrosis factor receptor, said kinase phosphorylates the p60 form of the tumor necrosis factor receptor. Also provided are various methods of manipulating this tumor necrosis factor receptor-associated protein and kinase in order to reduce various biological effects of tumor necrosis factor.

8 Claims, 33 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 33

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Drawing Description Text - DRTX (3):
FIG. 1 shows a schematic diagram of GST fusion proteins containing the cytoplasmic domain of the p60 form of the tumor necrosis factor receptor. The full-length p60 TNF receptor is shown with the extracellular domain (ED), the transmembrane region (shaded), and the cytoplasmic domain (CD). Residue numbering is based on the mature form of the receptor. Plasmids (left) and the

fusion proteins (right) expressed from them are indicated. GST-p60CD.DELTA.1 was used entirely. gg designates the glycine linker in the fusion protein GST-g-p60CD.

Detailed Description Text - DETX (13):

and were used to amplify a 671-bp fragment that encodes residues Y207 to R426 of p60. The PCR fragment was digested with EcoRI and XhoI and ligated into EcoRI/XhoI-digested pGEX-KG to give rise to pGEX-KG-p60CD. The pGEX-KG-p60CD was digested with EcoRI and partially digested with HindIII, and both the 700-bp (EcoRI/HindIII fragment) and the 570-bp (HindIII/HindIII due to an internal HindIII site in the p60 gene) fragments were isolated. The 700-bp EcoRI/HindIII fragment was inserted into EcoRI/HindIII-digested pGEX-2TH and termed pGEX-2TH-p60CD. In order to place the p60CD coding sequence in frame with GST, pGEX-2TH-p60CD was further digested with BamHI, filled in with Klenow, and religated to give rise to pGEX-2THAB-p60CD. Additionally, the 570-bp HindIII/HindIII fragment was inserted into HindIII-digested pGEX-2THAB to give pGEX-2THAB-p60CD.DELTA.1.

US-PAT-NO: 5843713

DOCUMENT-IDENTIFIER: US 5843713 A

TITLE: Peptide sequence that forms mucin sugar chain and technique for modifying protein to be linked with mucin sugar chain

DATE-ISSUED: December 1, 1998

INVENTOR-INFORMATION:

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APPL-NO: 08/ 666473

DATE FILED: September 19, 1996

FOREIGN-APPL-PRIORITY-DATA:

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| JP | 6-269111 | November 1, 1994 |
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PCT-DATA:
APPL-NO: PCT/JP95/02238
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PUB-NO: WO96/13516
PUB-DATE: May 9, 1996
371-DATE: Sep 19, 1996
102(E)-DATE:Sep 19, 1996

US-CL-CURRENT: 435/69.1, 435/320.1 , 435/325 , 435/70.1 , 536/23.1 , 536/23.4

ABSTRACT:

An amino acid sequence that can specifically introduce a mucin type sugar chain into a protein or peptide chain and a technique of introducing a mucin type sugar chain into protein or peptide by utilizing such a sequence are disclosed. GalNAc moiety of UDP-GalNAc (where UDP represents uridine 5'-diphosphate and GalNAc represents N-acetylgalactosamine) is introduced into the amino acid X(O) in the presence of UDP-GalNAc: polypeptide .alpha.1, O-GalNAc transferase (O-GalNAc T):

X(-1)-X(0)-X(1)-X(2)-X(3) (I)

where X(-1) and X(2) represent independently any amino acid, X(O) represents T or S and X(1) and X(3) represent independently any amino acid except that at least one of X(1) and X(3) represents P.

13 Claims, 26 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 23

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Drawing Description Text - DRTX (13):
FIG. 12 is a graph showing the amount of GalNAc transferred to GST-3X Muc C1 including a peptide sequence having GalNAc acceptor activity at the C-terminal region of protein GST and controls of protein having no such peptide sequence.

Drawing Description Text - DRTX (16):
FIG. 15 is a graph showing the amount of GalNAc transferred to GST-3X 2A Muc N1 including a peptide sequence having GalNAc acceptor activity at the N-terminal side of protein GST and controls of protein having no such peptide sequence.

Drawing Description Text - DRTX (17):
FIG. 16 illustrates a construction of expression plasmid pGEX-3XS which is used for the production of a GST mutant including a peptide sequence having GalNAc acceptor activity at the C-terminal side of protein GST.

Detailed Description Text - DETX (133):
As a model protein, a derivative of glutathione S-transferase (GST) from Schistosoma japanicum was used. The derivative of GST (GST-3X) can easily be prepared on a mass production basis from E. coli with commercially available plasmid pGEX-3X (Pharmacia Biotech). The derivative of GST had a peptide sequence SDLIEGRGIPGNSS added to the C-terminal of native GST. The gene of the protein contained in plasmid pGEX-3X was used.

Detailed Description Text - DETX (138):
Subsequently, 50 .mu.l of a solution containing 10 mM Tris-HCl (pH 8.0), 5 mM MgCl.sub.2, 100 mM NaCl, 1 mM 2-mercaptoethanol and 1 nmol of each of the above synthesized DNAs were prepared. The solution was then warmed to 75.degree. C. for 10 minutes and thereafter left to room temperature for annealing to produce a double-strand DNA, which was the desired DNA. A 5 .mu.l portion of the solution thus obtained was taken and the double-strand DNA was cut with EcoR I and BamH I and inserted between the same restriction enzyme sites of pGEX-3X to construct plasmid pGEX-3X Muc C1 according to a conventional method. The plasmid contained a DNA encoding the mutant, GST-3X Muc C1, n which MAAATPAPM was inserted between the 228th proline and the 229th glycine of the GST-3X. The sequence of the inserted region was confirmed by 373 A DNA sequencer (Applied Biosystems) with 5'pGEX Sequencing Primer (Pharmacia Biotech) and PRISM, Dye Terminator Cycle Sequencing Kit (Applied Biosystems).

Detailed Description Text - DETX (148):
The GST-3X gene in pGEX-3X does not have any restriction site in the N-terminal region for inserting a DNA fragment of a peptide sequence having a GalNAc acceptor activity. Therefore, a gene for GST-3X 2A having a restriction site of Nco I was prepared by polymerase chain reaction (PCR). At first, the following primers were synthesized with 394 DNA/RNA Synthesizer available from Applied Biosystems.

Detailed Description Text - DETX (151):
Then, the GST-3X 2A DNA was cut from pSL1190 by Nco I and Pst I, and inserted between the same restriction sites of pTrc99A (Pharmacia Biotech) to obtain pGEY-3X 2A. The plasmid pGEY-3X 2A is very similar to pGEX-3X except that it contains GST-3X 2A gene having an Nco I site at the N-terminal where a DNA can be inserted and that the promoter is switched from tac to trc.

Detailed Description Text - DETX (152):

Finally, a plasmid of pGEY-3X 2A Muc N1 containing GST-3X 2A Muc C1 gene having a peptide sequence of MAAATPAP (SEQ ID NO:104) at the N-terminal was prepared in a manner as described below. A gene coding for the peptide sequence MAAATPAP (SEQ ID NO:104) was prepared by synthesizing single-strand Synthesized DNA 1 and Synthesized DNA 2 same as those of Example 11 and annealing them in 50 .mu.l of a solution containing 50 mM Tris-HCl (pH 7.5), 10 mM MgCl.sub.2, 100 mM NaCl and 1 mM 2-mercaptoethanol. In 5 .mu.l of the solution thus obtained, the double-strand DNA was cut by Nco I and inserted into the Nco I site of pGEY-3X 2A to produce plasmid pGEY-3X 2A Muc N1. The plasmid has MAAATPAP (SEQ ID NO:106) upstream to the methionine at the N-terminal of GST-3X and contains a DNA coding for a mutant, GST-3X 2A Muc N1, in which the serine at the second position of GST-3X had been changed to alanine. The sequence of the inserted region was confirmed by 373A DNA Sequencer (Applied Biosystems) with 5'-GTTGACAATTAATCATCCGGCTCGT-3' ((SEQ ID NO:106) synthesized and purified with HPLC by Kurasiki-Bouseki) and PRISM, Dye Terminator Cycle Sequencing Kit (Applied Biosystems).

Detailed Description Text - DETX (182):

Since GST is an intracellular protein, genes of GST-3X and GST-3X Muc C1 to which a signal sequence for secretion was added to the N-terminal were prepared by a 2-step PCR process. The signal sequence of human erythropoietin (hEPO) [K. Jacobs et al., Nature, Vol.313, pp.806-810 (1985)] was used in a manner as described below.

Detailed Description Text - DETX (189):

A gene of a secretion form protein of GST-3X Muc C1 was prepared in the same manner as described above except that pGEX-3X Muc C1 was used instead of pGEX-3X. pBEGST-3X Muc C1 was thus obtained.

Detailed Description Text - DETX (201):

Example 14 showed that a GST mutant obtained by inserting a peptide sequence of MAAATPAPM (SEQ ID NO:105) was secretory expressed in COS7 cells and the produced EGST-3X Muc C1 had a typical mucin type sugar chain. Thus, in this example, each of GST-3X Muc C2, GST-3X Muc C3 and GST-3X Muc C4, that was confirmed to function, like GST-3X Muc C1, as a substrate for in vitro GalNAc transfer in Example 13, was fused with a signal peptide and secretory expressed in COS7 cells to confirm if the expressed proteins EGST-3X Muc C2, EGST-3X Muc C3 and EGST-3X Muc C4 can bind mucin type sugar chains. In addition, EGST-3X Muc C5 having a sequence of GTPGNSS, where amino acid at Position +1 is proline in the C-terminal region of EGST-3X, was also prepared.